CBI FORENSIC LABORATORY

DNA ANALYSIS SOP, VERSION 2.1

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DNA LABORATORY ORGANIZATION

The Colorado Bureau of Investigation (CBI) DNA Laboratory is organized according to the most recent guidelines established by the SWGDAM (Scientific Working Group on DNA Analysis Methods). Because of the sensitivity of PCR-based DNA analyses and the consequent potential for sample contamination, precautions must be implemented at each stage of the analytical process to avoid concurrent and future contamination. These sources as well as the appropriate measures to prevent such contamination follow.

- Sample contamination with human genomic DNA from the environment surrounding a sample. This chance contamination could be introduced with the analyst's own DNA during handling or by DNA from other specimens examined before the sample in question.
- Contamination between samples during sample preparation (from pipette tips, tools, aerosols). This is a specific type of environmental contamination and is of concern particularly when samples of high DNA concentration are prepared simultaneously with low level DNA samples.
- Contamination of a sample with amplified DNA from a previous PCR reaction, referred to as "PCR product carryover." Since the number of copies of amplified DNA in a completed PCR reaction is so high, the inadvertent transfer of even a minute volume to a sample yet to be amplified may result in the amplification and typing of the contaminating DNA sequence. This could lead to complete mischaracterization of the sample.

The DNA laboratories at the CBI have been organized in order to minimize the chance of any type of contamination. Recommendations developed by Cetus, TWGDAM, DAB, Roche Molecular Systems, and SWGDAM have proven effective in preventing these types of contamination and have been followed here. The area in which amplified DNA is handled is physically isolated from the areas for DNA extraction and PCR setup. CBI has two designated work areas for DNA analysis: (1) DNA extraction/PCR setup room and (2) PCR amplification and typing room(s).

The DNA extraction area is separated by time from the PCR setup area. All DNA extractions are performed in the PCR workstations equipped with both fluorescent and ultraviolet lamps. The PCR workstation is exposed to ultraviolet light for 20-30 minutes between DNA extraction and PCR setup, between extractions of evidence samples and standard samples, and between extractions of samples with minimal amounts of DNA and samples with high levels of DNA.

The PCR amplification and typing area is physically separated from evidence examination, DNA extraction, and PCR setup. Production and handling of amplified DNA is restricted to the separate PCR amplification and typing room(s).

EVIDENCE EXAMINATION WORK AREA

Evidence processing, including examination and sample collection, is performed in the Serology laboratory. Stain cards from database samples are prepared in a hood.

DNA EXTRACTION AREA

All DNA extractions are performed in the PCR workstations located in the DNA extraction/PCR setup room of the laboratory.

The following special precautions are strictly observed.

- Evidence samples are prepared at a separate time from standard samples.
- Samples with minimal amounts of DNA are prepared at a separate time from samples with high levels of DNA.
- Disposable gloves are worn during evidence examinations and analyses. Gloves are changed as necessary to avoid sample-to-sample contamination and when leaving the work areas.
- Sampling instruments are cleaned thoroughly with 10% bleach and deionized water between uses. A clean cutting surface is used (sheet of paper) for each specimen, and absorbent hood pads are replaced as necessary.
- Sampling is limited to that amount from which one might expect to obtain sufficient DNA as required by the investigation at hand.
- Those solutions, which can be heated in an autoclave without affecting their performance, are steam sterilized. This insures that any contaminating DNA is rendered unamplifiable.
- Sterilized microcentrifuge tubes and sterile aerosol resistant pipette tips are used.
- Aerosol resistant pipette tips are changed between each sample. They do not need to be changed when aliquotting kit reagents.
- Reagents are stored in aliquots as small as practical. This will allow for tracing of any contamination and minimize the number of times a given reagent is exposed to potential contamination.
- Tubes containing DNA in liquid are centrifuged before opening.
- Appropriate controls are always used. At a minimum, these controls include the positive amplification control, the negative amplification blank, and the appropriate reagent blanks and extraction controls (casework).
- DNA from casework samples is ultimately stored in screw-cap tubes.
- Equipment (centrifuges, pipettors, racks, etc.) is cleaned as needed.
- The PCR workstation is exposed to ultraviolet light for 20-30 minutes between preparation (cutting, removing, etc.) of evidence samples and standard samples, between extractions of samples with minimal amounts of DNA and samples with high levels of DNA, between DNA extractions and PCR setup, and before and after each use.
- Glassware is cleaned thoroughly and/or autoclaved prior to reagent storage.

- The quantity of samples handled in a single run is limited to a manageable number. This precaution reduces the risk of sample mix-up and the potential for sample-to-sample contamination.
- Clean, dedicated lab coats are worn at all times.

PCR SETUP AREA

PCR setup is performed in the PCR workstations located in the DNA extraction/PCR setup room of the laboratory. Setup of PCR reactions must be separated by time from other uses of the PCR workstation. This area is also used for dispensing PCR reaction mix aliquots into the PCR reaction tubes. The following special precautions apply while setting up PCR reactions (FTA samples exempt).

A. The PCR workstation must be irradiated with ultraviolet light for 20-30 minutes before setting up PCR reactions. Pipettors, vortexors, pipette tips, sampling instruments, labeling instruments, decappers, timers, and microtube racks remain in the PCR workstation during ultraviolet irradiation.

B. After addition of the PCR reaction mix to the tubes, all tubes are capped. After addition of the extracted DNA sample to the appropriate PCR reaction tube, the tube is recapped before proceeding to the next DNA sample.

C. Gloves are changed as necessary to avoid contaminating other specimens.

D. Kit reagents are handled and stored such that they are not exposed to extraneous DNA. Mineral oil is not to be exposed to be ultraviolet light, as this can cause the oil to inhibit PCR.

E. Microtube racks that are used for carrying the completed PCR reaction mix tubes to the thermal cycler in the PCR amplification and typing room(s) must be exposed to a minimum of 20 minutes of ultraviolet light per surface area before they are returned to the PCR workstation in the DNA extraction/PCR setup room.

PCR AMPLIFICATION AND TYPING AREA

Access to the PCR amplification and typing area is restricted to DNA analysts and supervisors. Production and handling of amplified DNA is restricted to the PCR amplification and typing rooms so that strict physical isolation of PCR product is maintained to avoid transfer of amplified DNA into preparatory areas. DNA amplification, DNA typing (hybridization, stringent wash, color development, gel electrophoresis and capillary electrophoresis) and waste disposal of amplified DNA solutions are carried out in this area. All equipment, glassware, and supplies within this room are dedicated to PCR amplification and typing. Equipment and supplies used to handle amplified DNA are not taken out of these rooms unless first decontaminated with a minimum of 20 minutes exposure to ultraviolet light per surface area. Amplified DNA samples are autoclaved in these rooms prior to disposal.

The following special precautions apply while working in the PCR amplification and typing room.

- Clean, dedicated lab coats are worn in the PCR amplification and typing room and they are disposed of in this room.
- Gloves are removed when leaving this work area to avoid the transfer of amplified DNA into other work areas.
- Gloves are changed whenever they may have become contaminated with amplified DNA in order to reduce the unnecessary dispersal of DNA around the work area.
- Absorbent pads are placed on bench tops and are changed as necessary.
- Removal of equipment, supplies, and waste material is accomplished by one of the following methods: (1) ultraviolet light exposure for a minimum of 20 minutes per surface area or (2) double bagged in autoclave bags and taken directly to the autoclave.

STERILIZATION

Autoclaves are used for treatment of wastes prior to disposal (such as PCR products and blood tubes from database samples) and for the sterilization of reagents and materials. Whenever possible, autoclaved materials are sterilized in the area where they are used or produced.

CLEANING AND DECONTAMINATION

If a lab bench is contaminated, the absorbent pads should be folded inward on themselves and placed into an autoclave bag. This autoclave bag should be double bagged and taken directly to the autoclave. The lab bench should then be washed with a 10% bleach solution followed with a 95% ethanol rinse. New absorbent pads should be placed on the surface.

If a thermal cycler becomes contaminated, all of the tubes should be removed and discarded. If using the 480 Thermal Cycler, the oil should be removed from all the wells using cotton tipped applicators. Completely clean the block and affected surfaces with a 10% bleach solution. Ultraviolet light treat all affected surface areas for at least 20 minutes per surface area using an UV light source. Rinse with 95% ethanol.

If other pieces of equipment become contaminated, comparable cleaning procedures should be followed.

EVIDENCE HANDLING

A clear, well-documented chain of custody is maintained from the time the casework evidence is first received at the CBI Laboratory until it is released from the Laboratory to the submitting agency. Each sample is labeled with a unique identifier in accordance with CBI policy. This includes all of the extracted DNA samples. All evidence and samples from evidence are to be received, collected, handled, sampled, and stored so as to preserve the identity, integrity, condition, and security of the item.

After a DNA analysis has been completed, the samples of amplified DNA and the amplifications of corresponding control samples are discarded in the PCR amplification and typing room(s). Strips are photographed and dried on non-absorbent film (i.e. sheet protectors). The strips, photographs, or copies thereof are kept in the specific case file. The microcentrifuge tubes containing the extracted DNA are placed into heat-sealed plastic bags and then placed in the DNA packet along with the remaining stain cards, swabs, cuttings, samples, and controls. The DNA packet is stored in the evidence freezer at the CBI Laboratory until it is returned to the submitting agency.

A well-documented chain of custody is also maintained for database samples. The remaining blood and blood tube are destroyed after a stain card is prepared. Stain cards are stored at CBI according to the preparation procedure utilized. No database samples are released from the CBI Laboratory.

PREPARATION OF SAMPLES FOR DNA ANALYSIS

PRESERVATION

Evidence materials must be stored, handled, and processed keeping in mind the potential for conventional serological and DNA analysis. Evidence will be stored according to existing preservation procedures in the Laboratory to insure that DNA genetic evidence as well as conventional serology evidence will be preserved while in custody at CBI.

General identification of the biological material is performed prior to DNA analysis. Evidence samples submitted are evaluated to determine the appropriateness for DNA analysis. Testing of evidence and evidence samples should be conducted to provide the maximum information with the least consumption of sample. It is necessary to preserve a portion of the sample for future analysis unless written permission to consume the entire sample is obtained.

The procedures in place at the CBI in regard to the conventional examination, sampling, and analysis of biological evidence are applicable also for DNA analysis of such evidence.

Some of the guidelines with special pertinence to DNA analysis of evidence are reiterated below.

A. Each item is examined on clean paper; generation of blood dust is minimized; and tools and work area are properly cleaned after each use. This eliminates the potential for one item to contaminate another through casual contact, especially in those instances where large differences in potentially recoverable DNA exist between items.

B. Sampling of evidence for conventional serological analysis must be performed with consideration of whether DNA analysis could be more informative or appropriate.

C. Prior to DNA processing, hair should be microscopically characterized.

MISCELLANEOUS EVIDENCE

In addition to blood, semen, and saliva evidence, PCR technology can be utilized for the analysis of many other potential evidence specimens such as cigarette butts, postage stamps and envelopes, dry bone, preserved autopsy specimens, etc.

MINIMAL EVIDENCE SUBMITTED FOR DNA ANALYSIS

Evidence submitted to the DNA section may be minimal in nature and completely consumed during analysis. In the event that, upon evaluating a case, the analyst feels that any item might be consumed in the analysis, the analyst shall not proceed with the analysis of those items. The District Attorney and the submitting agency in that specific case shall be notified in the report of the nature of the minimal evidence. The District Attorney and the submitting agency may also be notified by telephone of the issue.

Analysis of minimal evidence shall only proceed upon the written authorization of the District Attorney or when there is no named defendant. If there is any doubt as to the status of a defendant on any given case, the analyst should contact the investigator assigned to the case for specifics.

Once the District Attorney contacts CBI and informs the analyst in writing that defense has been notified of the situation, arrangements shall be made by the analyst and the defense representative to observe ***only the extraction of the DNA from the minimal evidence***. ***The defense representative will observe no further DNA analyses*.** If desired, the defense shall be given one-half of the extracted DNA to afford them the opportunity to repeat any analyses performed at CBI.

Under no circumstances shall the defendant's or prosecution's representatives be allowed to be present while DNA tests, which can be repeated, are being performed. This also includes all non-CBI personnel regardless of whether they are involved in the case. Any court orders or attempts by the defense to have their experts present during DNA analysis/typing shall be brought to the immediate attention of the Agent-in-Charge (AIC) so that the motion may be addressed in a suitable manner.

Under no circumstances shall the defendant's representatives or any outside experts be allowed to use any equipment or instrumentation owned by CBI.

QUALITY CONTROL MEASURES

REAGENTS

DNA analysis reagents are "reagent grade" or better. A log is maintained which records and inventories reagents, date chemicals/reagents received, supplier, catalog number, lot number, storage location, and expiration date, where appropriate. The in-house reagents are labeled as to content and concentration, lot # (date of preparation and initials of preparing analyst), and expiration date (expiration date must be present; if no expiration date is required, then write "No expiration date"). A log is maintained for in-house reagents which records date of preparation, the amount made, lot numbers of chemicals used, the preparer, and an expiration date. Formulation procedures for reagents, standards, and controls are also included at the top of each reagent log form, and in the CBI Forensic Laboratory DNA SOP Manual.

A log is maintained of commercial amplification kits, quantitation kits.

CRITICAL REAGENTS

The DNA Laboratory identifies critical reagents and verifies them prior to use in casework and database analyses. These critical reagents include:

- commercial kits for performing genetic typing for casework analysis,
- reagents used in the extraction of casework samples, and
- FTA paper for database

Amplification reagents are commercially prepared and supplied with the Amplitype®, AmpFℓSTR Profiler Plus, AmpFℓSTR Cofiler kits, and AmpFℓSTR Identifiler kits. They are quality controlled by the manufacturer prior to release for sale. In this Laboratory, their performance, as well as the performance of in-house reagents, is monitored with each extraction, amplification, and typing run by the use of a known reagent blank, positive control, and negative amplification blank which are outlined below. The integrity of the components of each Amplitype®, AmpFℓSTR Profiler Plus, AmpFℓSTR Cofiler kit, and AmpFℓSTR Identifiler kits, as well as the in-house prepared reagents, is maintained by strict adherence to laboratory procedures designed to prevent contamination of reagents. Verification of a manufacturer's lot for each type of kit used for DNA amplification will be performed using a NIST traceable control prior to use in casework analysis.

STANDARDS AND CONTROLS

The following controls and standards are employed in the PCR procedures.

- There are separate locations for pre- and post-amplification procedures. DNA extraction and PCR setup are separated by time.

- The preparation of known and questioned samples for DNA extraction is performed at different times. The extraction of minimal samples is also performed at different times from other items.
- Reagent blanks are included with each extraction to monitor the integrity of the extraction reagents and equipment. This blank is carried through the amplification and typing stages.
- Substrate controls are included when necessary to assess the background contribution potential of evidence stain substrates. These specimens are processed with evidence samples and are required when there is reasonable expectation of detectable background DNA in an evidence specimen.
- A negative amplification blank is required with each set of amplifications. This control demonstrates the integrity of the amplification kit components, as well as serving as a check for contamination during PCR reaction setup. It consists of a PCR cocktail to which sterile deionized water or sterile TE Buffer instead of DNA is added.
- A positive DNA control is required with each set of amplifications to test for success of amplification and to demonstrate that all aspects of the PCR reaction process are performing as expected in terms of specificity and sensitivity. The manufacturer supplies this control as a kit component or a NIST traceable standard that is extracted at the same time as the samples may be used.
- CASEWORK: A tissue extraction control is a blood sample that is a NIST traceable standard with a known DNA profile. It is extracted, quantitated, amplified, and typed with each set of blood, buccal, and tissue samples. The tissue extraction control may also be used to verify commercial kits, extraction reagents, thermal cyclers, and typing instruments.
- CASEWORK: A differential extraction control is a sperm/non-sperm mix sample that is a NIST traceable standard with a known DNA profile. It is extracted, quantitated, amplified, and typed with each set of sperm samples. The differential extraction control may also be used to verify commercial kits, extraction reagents, thermal cyclers, and typing instruments.
- DATABASE: The Internal DNA control is a blood sample that has a known DNA profile and is a NIST traceable standard. This control is also used to verify commercial kits, extraction reagents, thermal cyclers, and typing instruments.
- Human DNA quantitation by QuantiBlot® is required on all samples and tissue extraction controls that have been extracted for casework analysis.
- Reverse dot blots are examined for the presence of the control (C and S) dot for interpretation.
- A second analyst performs a technical review on all typing results.

EQUIPMENT CALIBRATION AND MAINTENANCE

The following equipment controls are employed in the PCR procedures. All procedures mandated by the *CBI Forensic Laboratory Quality Assurance Manual* are followed.

THERMAL CYCLER

A. The Temperature Calibration Verification Test of the thermal cycler is performed biannually using a calibrated temperature verification kit.

B. The Temperature Uniformity Test of the thermal cycler is performed biannually using a calibrated temperature verification kit.

C. The Perkin-Elmer 480 thermal cycler sample block is cleaned after each use with cotton swabs.

D. The temperature verification kit is sent out annually for recalibration.

E. Each thermal cycler used for amplification is verified with a NIST traceable standard annually.

HYBRIDIZATION BATHS AND HEATING BLOCKS

A. The hot shaker water bath(s) and heating block(s) temperatures are checked prior to use and monitored during use with a NIST traceable thermometer.

B. The NIST traceable thermometer is sent out for recalibration annually.

PIPETTORS

Pipettors are sent to a contractor annually for cleaning, calibration, and general maintenance.

CENTRIFUGES

Centrifuges are cleaned and serviced as necessary.

pH METER

The pH meter is calibrated prior to each use with the appropriate buffer(s) of known pH.

BALANCES

Balances are serviced and recalibrated annually according to service contract specifications. They are checked in-house monthly by weighing known standard weights and comparing the displayed value to the known value.

WATER PURIFICATION SYSTEMS

Water purification systems are maintained and are sanitized as needed.

REFRIGERATORS, FREEZERS (-20^{O}C AND -70^{O}C), AND INCUBATORS

Refrigerators, freezers (-20^{o}C and -70^{o}C), and incubators are maintained and temperatures are taken on a weekly basis. Temperatures are logged on a temperature log sheet specific for each piece of equipment.

ABI 377™, ABI 310™, AND ABI 3100™

The ABI 377™, ABI 310™, and ABI 3100™ are maintained. Each instrument is verified annually with a NIST traceable standard.

MATERIAL SAFETY DATA SHEETS (MSDS)

MSDS have been obtained for all chemicals handled in the DNA laboratory. The MSDS are maintained in a binder and are updated as needed. Each analyst is aware of the binder's existence and location.

LOGBOOKS FOR CHEMICALS, COMMERCIAL KITS, IN-HOUSE REAGENTS, AND CRITICAL REAGENTS

Logbooks of lot numbers, expiration dates, and verifications of lots for chemicals, commercial kits for DNA typing, in-house reagents, and critical reagents are kept in a readily accessible area.

RECORDS OF EQUIPMENT TEMPERATURES, MAINTENANCE, AND CALIBRATION

Records of equipment temperature, maintenance, and calibration are kept in a logbook in a readily accessible area.

PROFICIENCY TESTING

Proficiency testing is used to demonstrate the quality of performance in the DNA Laboratory and serves as a mechanism for critical self-evaluation. This is accomplished by the analysis and reporting of results from appropriate biological specimens submitted to the Laboratory from an outside source of proficiency tests and also from specimens prepared in-house.

All specimens submitted as part of a proficiency test must be analyzed at all 13 CODIS STR loci. Additional loci can be added if analyzed (Identifiler). Interpretation of the results will follow current *CBI Forensic Laboratory DNA Analysis SOP* protocol for casework. The analyst will transmit the results of the proficiency test to the Quality Assurance Manager (QAM) or designee. The analyst will maintain the original data, calculations, and other information in a notebook, with a copy of the results to be given to the QAM or designee. All guidelines written in the *CBI Forensic Laboratory Quality Assurance Manual* are followed and the test handled as if it were evidence. A memo from the QAM or designee is given to the analysts regarding the results of their testing. Any discrepancies within a proficiency test are handled according to the *CBI Forensic Laboratory Quality Assurance Manual*.

When the consensus report is issued from the supplier of the proficiency test, the Technical Leader will review the report and then compare their results to those of each analyst who participated in the test. After the comparison, the Technical Leader will initial the analyst's proficiency test showing compliance to the consensus report. A memo, which can be in electronic form, will be sent to the QAM from the Technical Leader that addresses the evaluation of all participants in the proficiency test. Any discrepancies between the analyst and the consensus will be handled according to the *CBI Forensic Laboratory Quality Assurance Manual*.

TESTING REQUIREMENT PRIOR TO CASEWORK

Following the *CBI Forensic Laboratory Biological Science Training Plan*, each analyst will be required to successfully complete DNA analysis on a minimum of 40 blood samples and a range of samples typically encountered in forensic casework prior to conducting independent DNA casework. In addition, each analyst will be required to successfully complete one proficiency (qualifying) test.

PERIODIC TESTING REQUIREMENT

Each analyst who conducts DNA testing on casework specimens will be required to complete a minimum of one external proficiency test every 183 days to demonstrate the reliability of the Laboratory's analytical methods as well as the interpretive capability of the analyst.

TESTING REQUIREMENT PRIOR TO DATABASE ANALYSIS

Following the *CBI Forensic Laboratory Biological Science Training Plan*, each analyst will be required to successfully complete DNA analysis on a minimum of 40 blood samples prior to conducting independent DNA database analyses. In addition, each analyst will be required to successfully complete one proficiency (qualifying) test.

PERIODIC TESTING REQUIREMENT

Each analyst who conducts DNA testing on database specimens will be required to complete one external proficiency test every 183 days to demonstrate the reliability of the Laboratory's analytical methods as well as the interpretive capability of the analyst.

CODIS AND CODIS SEARCHES

CODIS, **Co**mbined **D**NA **I**ndex **S**ystem, is a database that was developed by the FBI to track the results of the DNA analyses performed on forensic specimens and convicted offenders. Accessibility to CODIS is limited to NDIS (**N**ational **D**NA **I**ndex **S**ystem) qualified personnel. CODIS security is maintained by keeping access to the server limited to CODIS Administrators. The server is located in a secure, locked room. Access to CODIS software, programs, and data is limited to NDIS qualified personnel and is password protected. Specimen identification is coded prior to entry into any CODIS database.

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DNA

PREPARATION AND STORAGE OF SAMPLES FOR CONVICTED OFFENDER DATABASE

SAMPLE SUBMISSION REQUIREMENTS

Requirements

Samples

A. Blood samples must consist of at least one Lavender (purple top) Vacutainer (EDTA) tube from each offender.

B. Buccal samples must be dried on the appropriate substrate and then sealed in a protective package.

Labeling and Packaging

A. The blood tube will be labeled with the offender's name (first and last), submitting agency case number, date the blood was drawn, and the initials of the person collecting the sample.

B. The protective packaging containing the buccal sample will be labeled with the offender's name (first and last), agency case number, and the date the sample was collected.

Storage Before Submitting Sample to CBI

A. Blood samples are required to be refrigerated in order to keep the samples cold at all times and submitted to the CBI Denver Laboratory within one week of collection. Blood should not have been frozen at any time.

B. The buccal sample should be thoroughly dried and placed in a protective package kept at room temperature.

Submission

The CBI Database Submission form must accompany a sample. The form will be kept separate from the blood tube or buccal sample and its sealed container.

CBI HANDLING OF DATABASE SAMPLES

Submission

A. At the time of sample submission to the CBI Denver Laboratory, CBI personnel will sign the chain of custody and assign a CBI case number. Database personnel will then enter the submission information into a catalog database, assign each sample a confidential PCR number, and dispense the necessary paperwork. The samples will be stored appropriately.

B. If the sample does not meet the requirements described in the Submission section, CBI personnel will use discretion as to whether or not the sample will be analyzed.

C. If a blood tube is broken or leaking at the time of submission to CBI, the sample will not be accepted.

Preparation of Stain Card From Liquid Blood

A. A stain card will be prepared for each blood sample. Each stain card will contain the offender's name, agency case number, CBI case number, and a unique PCR number assigned by CBI personnel.

B. Preparation of bloodstain cards will be carried out in a manner to minimize risk of contamination.

1. Handle the cards wearing gloves. Change gloves between samples when spotting the blood sample or when contact with blood may have occurred.
2. Prepare each new stain card on a clean surface.
3. Use a new tube decapper or fresh Kimwipe for each blood sample.
4. Do not allow prepared stain cards to come in direct contact with each other or with unprepared cards.

C. Upon completion of preparing the stain card, the offender's blood tube and any remaining blood will be sterilized by autoclaving followed by disposal in the biohazard trash.

D. When the spotted stain card is thoroughly dry, the card will be packaged in a plastic sleeve, heat-sealed with initials and date, and stored appropriately. The FTA Cards™ are stored locked at room temperature. The S & S™ cards are stored in a locked –70ºC freezer.

Storage of Buccal Samples

Buccal samples on cotton swabs are stored in the –70º freezer. Buccal samples on FTA cards are stored locked at room temperature.

PREPARATION OF REAGENTS

Before starting, UV treat all equipment and glassware 20 minutes per surface area or autoclave.

A. BSA (8 µg/µl BOVINE SERUM ALBUMIN)

Add 8 g BSA (Sigma #A2153) per every 1 L (0.8 g per every 100 ml) autoclaved ultrapure water. Mix thoroughly. Sterile filter through a Nalgene 0.2 or 0.45 micron filter unit. Aliquot into sterile 15 ml tubes. Aliquot from 15 ml tube into sterile 1.5 ml Sarstedt tubes as needed. Store all tubes at -20°C.

B. CHELEX™ SOLUTION 20% and 5% w/v (100 ml)

1. 20% (w/v) Chelex™ Solution*. Add 20 g Chelex™ to 100 ml sterile ultrapure H_2O. Aliquot into sterile 1.5 ml tubes.** Check that pH is greater than 9. Store at room temperature protected from light by covering the test tube rack with foil.

2. 5% (w/v) Chelex™ Solution. Add 5 g Chelex™ to 100 ml sterile ultrapure H_2O. Aliquot into sterile 1.5 ml tubes.** Check that pH is greater than 9. Store at room temperature protected from light by covering the test tube rack with foil.

*Needed for sperm-containing samples only.

** When pipetting Chelex™ Stock Solutions, the resin beads must be distributed evenly in solution; this can be achieved by gentle mixing with a stir bar in a beaker. Also, the pipette tip used must have a relatively large bore — 1ml tips are adequate.

C. 10X CITRATE BUFFER (1M SODIUM CITRATE, pH 5.0) (1L)

Dissolve 368 g trisodium citrate, dihydrate ($Na_3C_6H_5O_7 \bullet 2H_2O$) in 1600 ml ultrapure H_2O. Adjust pH to 5.0 (± 0.2) using approximately 60 g citric acid monohydrate ($C_6H_8O_7 \bullet H_2O$). Bring up to 1 liter with ultrapure water, mix, and autoclave. Store at room temperature.

D. CITRATE BUFFER (0.1M SODIUM CITRATE, pH 5.0) (1L)

Add 100 ml 10X Citrate Buffer to 900 ml ultrapure water. Store at room temperature.

E. DEIONIZED FORMAMIDE (50 ml)

Mix 50 ml formamide and 5 g ion-exchange resin. Stir for 30 minutes at room temperature. Check that the pH is greater than 7. If the pH is less than 7, decant the formamide into a beaker containing another 5 g ion-exchange resin and repeat the 30-minute stirring at room temperature. Allow the resin to settle. Filter the formamide and aliquot into 1.5 ml vials. Store up to 3 months at -20°C. (Optionally, use manufactured pre-deionized formamide, aliquot, and store at -20ºC.)

F. DIGEST BUFFER: 10mM Tris-HCl – 10mM EDTA – 50mM NaCl – 2% SDS, pH 7.5 (100 ml)

Mix together 1ml 1M Tris-HCl, pH 7.5; 2 ml 0.5M EDTA; 1ml 5M NaCl; 10 ml 20% (w/v) SDS; and 86 ml sterile ultrapure water. Store at room temperature.

G. 1M DITHIOTHREITOL, 10mM SODIUM ACETATE, pH 5.2 (5 ml)

Dissolve 0.77 g dithiothreitol in 5 ml sterile ultrapure water. Add 50 µl 1M sodium acetate pH 5.2. Do not autoclave. Aliquot (recommended 1 ml) and store at -20ºC.

H. 0.5M EDTA, pH 8.0 (1L)

Slowly add 186.1 g disodium ethylenediaminetetraacetic acid dihydrate ($Na_2EDTA \bullet 2H_2O$) to 800 ml ultrapure water. Stir vigorously on a magnetic stirrer. Adjust to pH 8.0 by adding NaOH pellets (approximately 20 g). Adjust final volume to 1 liter with ultrapure water. Sterilize by autoclaving. Store at room temperature.

NOTE: EDTA will not go into solution without pH adjustment.

I. 200mM EDTA (10 ml)

Add 4.0 ml 0.5M EDTA, pH 8.0 to 6.0 ml of sterile ultrapure H_2O and mix thoroughly. The solution may be aliquotted, 300 µl/tube, into microcentrifuge tubes. Store at 2° to 8ºC.

J. HYBRIDIZATION SOLUTION (5X SSPE, 0.5% w/v SDS) (1 L)

Add 250 ml 20X SSPE and 25 ml 20% w/v SDS to 725 ml ultrapure water and mix thoroughly. Hybridization Solution solids must be in solution before use; warm in an incubator to 50-55ºC to dissolve solids completely prior to use. Preparation in a clear glass container is recommended to facilitate visual inspection for solids during warming.

K. 3% HYDROGEN PEROXIDE (1 ml)

Add 1 ml 30% H_2O_2 to 9 ml sterile ultrapure water and vortex to mix. Protect from light. Store at 2° to 8ºC. The 3% hydrogen peroxide has a shelf life of approximately 4 weeks.

L. 30% H_2O_2 (5 ml Aliquots)

Aliquot 5 ml into 15 ml conical tubes, wrap with Parafilm®, and store at 4ºC in QuantiBlot® area.

M. ORGANIC SPERM WASH BUFFER (10mM Tris-HCl – 10mM EDTA – 50mM NaCl – 2 % SDS, pH 8.0, 500 ml)

Add 5 ml 1M Tris-HCl, pH 8.0; 10 ml 0.5M EDTA (pH 8.0); 5 ml 5M NaCl; and 50 ml 20% SDS to 430 ml ultrapure water (or use 0.29 g NaCl in place of the 5 ml 5M NaCl) and adjust volume to 500 ml. The pH should be adjusted to 8.0. Autoclave. Store at room temperature.

N. ORGANIC STAIN EXTRACTION BUFFER (10mM Tris – 100mM NaCl – 10mM EDTA – 2% SDS, 1L)

Dissolve 5.84 g NaCl in 500 ml ultrapure water with stirring. To this solution add 10 ml 1M Tris, pH 8.0; 20 ml 0.5M EDTA; and 100 ml 20% SDS. Titrate to pH 8.0 with HCl. Bring to a final volume of 1L with ultrapure water. Autoclave. Store at room temperature.

O. PBS BUFFER, pH 7.4 (PHOSPHATE BUFFERED SALINE): 2.7mM KCl – 137mM NaCl – 1.5mM KH_2PO_4 – 8.0mM Na_2HPO_4, pH 7.4 (1L)

Dissolve 0.2 g KCl, 8.0 g NaCl, 0.2 g KH_2PO_4, and 2.2 g $Na_2HPO_4 \bullet 7H_2O$ (or 1.1 g Na_2HPO_4, anhydrous) in 800 ml ultrapure water. Adjust pH of solution to 7.4 if necessary. Adjust to final volume of 1 liter using ultrapure water. Sterilize by autoclaving. Store at room temperature.

P. P/C/I Phenol/chloroform/isoamyl alcohol (25:24:1; v/v)

Phenol/chloroform/isoamyl alcohol saturated with buffer to a pH of 8.0 ± 0.5 is used for the purification of DNA.

CAUTION: This solution is an irritant and is toxic – it must be confined to a fume hood.

Q. PM + DQA1 WASH SOLUTION (2.5X SSPE, 0.1% w/v SDS) (2L)

Add 250 ml 20X SSPE and 10 ml 20% w/v SDS to 1,740 ml ultrapure water and mix thoroughly. Store at room temperature. Wash solution solids must be in solution and the solution must be well mixed before use; warm in an incubator to 50-55ºC to dissolve solids completely, and prior to use. Preparation in a glass container is used to facilitate visual inspection for solids during warming.

R. PRE-WETTING SOLUTION (0.4N NaOH, 25mM EDTA) (500 ml)

Add 20 ml 10N NaOH, 25 ml 0.5M EDTA to 455 ml ultrapure water and mix thoroughly. Store at room temperature.

S. 10 mg/ml PROTEINASE K (10 ml)

Dissolve 100 mg Proteinase K in 10 ml sterile ultrapure water. Aliquot solution (0.5 ml) and store 0.5 ml aliquots frozen at -20ºC.

T. QUANTIBLOT® WASH SOLUTION (1.5X SSPE, 0.5% w/v SDS) (2L)

Add 150 ml 20X SSPE and 50 ml 20% w/v SDS to 1,800 ml ultrapure water and mix thoroughly. Wash solution solids must be in solution before use; warm to 50-55ºC in an incubator to dissolve solids completely prior to use. Prepare in a glass container to facilitate visual inspection for solids during warming.

U. 20% SARKOSYL (N-Laurylsarcosine, 100 ml)

Add 20 g N-Laurylsarcosine ($C_{15}H_{28}NO_3Na$) to ultrapure water and stir until dissolved. Bring to a final volume of 100 ml with sterile ultrapure water.

V. 1M SODIUM ACETATE, pH 5.2 (100 ml)

Dissolve 13.6 g $CH_3COONa \bullet 3H_2O$ in 80 ml ultrapure water. Adjust to pH 5.2 by adding glacial acetic acid (approximately 2 ml). Adjust the final volume with ultrapure water to 100 ml. Sterilize by autoclaving. Store at room temperature.

W. 5 M SODIUM CHLORIDE (1 L)

Dissolve 292.2 g NaCl in 800 ml ultrapure water. Adjust final volume with ultrapure water to 1 liter. Sterilize by autoclaving. Store at room temperature.

X. 20% (w/v) SDS (SODIUM DODECYL SULFATE) (lL)

CAUTION: Wear protective mask when weighing SDS

Slowly dissolve 200 g electrophoresis-grade (ultra-pure) sodium dodecyl sulfate (SDS) in 800 ml sterile ultrapure water. Warming (e.g. in a 37°C water bath) may be required to dissolve solids completely. Adjust volume to 1L with sterile ultrapure water and mix thoroughly. Store at room temperature.

Y. SPOTTING SOLUTION (0.4N NaOH, 25mM EDTA, 0.00008% BROMOTHYMOL BLUE) (75 ml)

Add 3 ml 10N NaOH, 3.75 ml 0.5M EDTA, and 150 µl 0.04% Bromothymol Blue (provided in QuantiBlot® Kit) to 68 ml ultrapure water and mix thoroughly. Spotting Solution is stable for three months when stored at room temperature.

Z. 20X SSPE BUFFER [3.6M NaCl, 200mM($NaH_2PO_4 \bullet H_2O$), 20mM EDTA, pH 7.4] (1L)

Dissolve 14.8 g disodium ethylenediaminetetraacetic acid dihydrate in 1600 ml ultrapure water. Adjust the pH to 6.0 (± 0.2) with 10N NaOH solution. Add 420 g sodium chloride (NaCl) and 55.2 g sodium phosphate, monobasic, monohydrate ($NaH_2PO_4 \bullet H_2O$). Adjust the pH to 7.4 (± 0.2) with 10N NaOH (about 10 ml). Adjust the final volume to 1 liter with ultrapure water and mix thoroughly. Autoclave and store at room temperature.

AA. STERILE SALINE

Dissolve 8.5 g NaCl in 1000 ml sterile ultrapure water. Autoclave.

BB. TBE BUFFER (89mM TRIS-BORATE — 2.0mM EDTA, pH 8.3)

1. 10X TBE CONCENTRATED STOCK: 0.89M TRIS-BORATE – 0.020M EDTA (1 L)

   To 40 ml 0.5M EDTA, pH 8.0, add approximately 800 ml ultrapure water. Add 108 g Tris base and 55 g Boric Acid to the diluted EDTA solution. Stir vigorously on a magnetic stirrer. Adjust volume to 1L with ultrapure water. Filter using a 0.2 or 0.45 micron Nalgene filter unit. Store at room temperature.

2. 1X TBE WORKING STOCK

   Immediately before use, dilute one part 10X Concentrated TBE Stock with nine parts ultrapure water.

CC. TE BUFFER: 10mM Tris-HCl — 0.1mM EDTA, pH 8.0 (1L)

Mix together 10 ml 1M Tris-HCl, pH 8.0; 0.2 ml 0.5M EDTA; and 990 ml ultrapure water. Sterilize by autoclaving. Store at room temperature.

DD. TRIS/EDTA/NaCl (10mM Tris-HCl – 100mM NaCl – 1mM EDTA, pH 8.0, 100 ml)

Add 1ml 1M Tris-HCl to approximately 75 ml ultrapure water. To this solution, add 0.584 g NaCl and 200 μl 0.5M EDTA. Stir until dissolved. Adjust the pH to 8.0 with 1N NaOH and bring to a final volume 100 ml with ultrapure water. Autoclave and store at room temperature.

EE. 1M TRIS-HCl, pH 7.5 (1L)

1. Dissolve 121.1 g Tris base in 800 ml ultrapure water. Adjust to pH 7.5 at room temperature by adding concentrated HCl (approximately 65 ml).

NOTE: Electrodes do not accurately measure the pH of Tris buffer; be sure to use suitable electrodes for the Tris buffer pH adjustment.

2. Adjust the final volume to 1 liter with ultrapure water. Sterilize by autoclaving. Store at room temperature.

FF. 1M TRIS-HCl, pH 8.0 (1L)

1. Dissolve 121.1 g Tris base in 800 ml ultrapure water. Adjust to pH 8.0 at room temperature by adding concentrated HCl (approximately 45 ml).

NOTE: Electrodes do not accurately measure the pH of Tris buffer; be sure to use suitable electrodes for the Tris buffer pH adjustment.

2. Adjust the final volume to 1 liter with ultrapure water. Autoclave. Store at room temperature.

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INORGANIC EXTRACTION (CHELEX™)

All extraction steps must be performed in the DNA extraction/PCR setup laboratory using hoods, reagents, and pipettors dedicated to this area.

Chelex™ is a chelating resin that has a high affinity for polyvalent ions. The Chelex™ resin is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions, which act as chelating groups. It has been postulated that the presence of Chelex™ during boiling prevents the degradation of DNA by chelating metal ions that may catalyze the breakdown of DNA subjected to high temperatures in low ionic strength solutions. The basic Chelex™ procedure consists of boiling the sample in a 5% Chelex™ solution, and then adding a fraction of the supernatant directly to the PCR Mix. This Chelex™ procedure results in denatured sample DNA.

BLOOD

PROCEDURE

A. Add about a 3 mm square of bloodstain or 3 µl whole blood to a sterile 1.5 ml microcentrifuge tube. Add 1 ml of TE Buffer.

B. Incubate at room temperature for 15 to 30 minutes. Mix occasionally by inversion or gentle vortexing.

C. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

D. Carefully remove supernatant (all but 20 to 30 µl) and discard. If the sample is a bloodstain, leave the fabric substrate in the tube with the pellet.

E. Add 5% Chelex™ to a final volume of 200 µl.

F. Incubate at 56°C for 15 to 30 minutes or overnight. (Overnight is optimal.)

G. Vortex at high speed for 5 to 10 seconds. Spin in a microcentrifuge for 5 to 10 seconds at 10,000 to 15,000 x g.

H. Incubate in a boiling water bath for 8 minutes.

I. Vortex at high speed for 5-10 seconds.

J. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

K. The sample is now ready for the PCR Quantification and Amplification process.

L. Store the remainder of the supernatant at 2° to 8°C or frozen. To re-use, repeat ***Steps I through K***.

SPERMATOZOA EXTRACTION IN THE PRESENCE OF CONTAMINATING CELLS (POST COITAL)

A. Dissect swab or fabric. Use a clean cutting surface for each different sample.

Steps B through G optional

B. Pipet 150 to 500 µl of PBS into a sterile 1.5 ml microcentrifuge tube. Add swab or fabric cutting.

C. Incubate at 4°C for 2 hours or overnight.

D. Twirl the swab or fabric with a sterile pick (applicator stick) for at least 2 minutes to agitate the cells off the substrate.

E. Remove the swab or fabric with the pick. It is advisable not to discard the substrate until microscopic analysis (***Step P***) shows that the sample contains sperm. Store swab or fabric in a sterile tube.

OPTIONAL: Transfer swab or fabric into a Costar spin basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at maximum speed for five minutes. Remove basket insert from extract tube. Remove the extracted material from the basket, place in a clean microfuge tube, and freeze if required. Otherwise, discard.

F. Centrifuge the sample in a microcentrifuge for 1 minute at 10,000 to 15,000 x g at room temperature. (A Costar spin basket may be utilized for the agitation of the cellular component from the substrate).

G. Without disturbing the pellet, remove all but 50 µl (or twice the volume of the pellet, whichever is greater) of the supernatant using a sterile Pasteur pipette or the tip of a sterile 1 ml disposable pipette and place into the tube containing the swab or fabric. This supernatant can be used for traditional serological analyses if necessary. Re-suspend the pellet in the remaining 50 µl by stirring it with a sterile pipette tip. This pellet contains epithelial cells and sperm cells and is called the cell debris pellet.

H. OPTIONAL: Remove about 3 µl of the re-suspended sample and spot on a glass microscope slide for examination. Stain with Christmas Tree Stain (refer to the *CBI Forensic Laboratory Serology SOP*: "Staining of Spermatozoa:Kernechtrot/ Picroindigocarmine").

NOTE: If epithelial cells are detected, proceed with the differential lysis procedure beginning with Step I. If no epithelial cells are observed, the differential lysis procedure may be omitted and the sample may be processed beginning with Step Q.

I. To the approximately 50 µl of re-suspended cell debris, add TE Buffer to a final volume of 200 µl. Add 2 µl of 10 mg/ml Proteinase K. Mix gently.

J. Incubate at 37°C for about 1 hour to lyse epithelial cells, but for no more than 2 hours, to minimize lysis of sperm.

K. Spin in a microcentrifuge for 5 minutes at 10,000 to 15,000 x g.

L. Add 150 µl of the supernatant to 50 µl of 20% Chelex™ in a fresh 1.5 ml microcentrifuge tube. Save for epithelial DNA analysis, beginning with ***Step S***.

M. Wash the sperm pellet as follows. Re-suspend the pellet in 0.5 ml DIGEST BUFFER. Vortex briefly. Spin in a microcentrifuge for 5 minutes at 10,000 to 15,000 x g. Remove all but 50 µl of the supernatant and discard the supernatant.

N. Repeat wash ***Step M*** an additional 2 times.

NOTE: Additional wash steps are recommended when the ratio of sperm to epithelial cells is low.

O. Wash once with sterile distilled water as follows. Re-suspend the pellet in 1ml sterile water. Vortex briefly. Spin in a microcentrifuge for 5 minutes at 10,000 to 15,000 x g. Remove all but about 50 µl of the supernatant, and discard the supernatant.

P. Re-suspend the pellet by stirring with a sterile pipette tip. Remove about 3 µl of the re-suspended sample and spot on a glass microscope slide for cell examination. Stain with Christmas Tree Stain (refer to the *CBI Forensic Laboratory Serology SOP: Staining Of Spermatozoa: Kernechtrot/Picroindigocarmine)*. (***Steps I to K, M to P*** may be repeated if nucleated or non-nucleated non-sperm cells are detected in the cell examination).

Q. Add about 150 µl of 5% Chelex™ to the approximately 50 µl re-suspended sperm cell pellet (final volume should be about 200 µl). Add 2 µl of 10 mg/ml Proteinase K and 7 µl of 1M DTT. Mix gently.

R. Incubate at 37°C for 30 minutes to 1 hour.

S. Vortex epithelial and sperm cell samples at high speed for 5 to 10 seconds.

T. Spin in a microcentrifuge for 10 to 20 seconds at 10,000 to 15,000 x g.

U. Incubate the samples in a boiling water bath for 8 minutes.

V. Vortex at high speed for 5 to 10 seconds.

W. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

X. The samples are now ready for the PCR quantification and amplification process.

Y. Store the remainder of the supernatant at either 2° to 8°C or frozen. To re-use, repeat ***Steps V*** through ***X.***

SEMEN

WHOLE SEMEN

A. Add 3 µl of whole semen to 200 µl of 5% Chelex™ in a 1.5 ml microcentrifuge tube.

B. Add 2 µl of 10 mg/ml Proteinase K and 7 µl of 1 M DTT. Mix gently.

C. Incubate at 56°C for 30 to 60 minutes. Vortex at high speed 5 to 10 seconds.

D. Spin in a microcentrifuge for 10 to 20 seconds at 10,000 to 15,000 x g.

E. Follow protocol for Whole Blood/Blood Stains beginning with ***Step H***.

SEMEN STAINS

A. Dissect stain material. Use a clean cutting surface for each different sample.

B. Pipet 1ml of PBS into a sterile 1.5 ml microcentrifuge tube. Add fabric cutting.

C. Incubate at room temperature for 30 minutes.

D. Vortex the tube at high speed for 1 minute. Remove the fabric with a toothpick or pipette tip. (A Costar spin basket may be utilized for the agitation of the cellular component from the substrate. See page 13, E – OPTIONAL)

E. Spin in a microcentrifuge for 2 minutes at 10,000 to 15,000 x g.

F. Without disturbing the pellet, remove and discard all but 50 µl of the supernatant using a microtip disposable transfer pipette. Re-suspend the pellet in the remaining 50 µl by stirring with a sterile pipette tip.

G. OPTIONAL: Remove about 3 µl of the re-suspended sample and spot on a glass microscope slide for examination. Perform Christmas Tree Stain (Serology SOP: Staining of Spermatozoa:Kernechtrot/ Picroindigocarmine).

NOTE: If epithelial cells are detected, follow the post-coital protocol beginning with Step I. If not detected, proceed as follows.

H. To the approximately 50 µl re-suspended cell pellet, add 5% Chelex™ to a final volume of 200 µl. Add 2 µl of 10 mg/ml Proteinase K and 7 µl of 1M DTT. Mix gently.

I. Incubate at 56°C for 30 to 60 minutes. Vortex at high speed for 5 to 10 seconds.

J. Spin in a microcentrifuge for 10 to 20 seconds at 10,000 to 15,000 x g.

K. Follow protocol for Blood/bloodstains beginning with ***Step H***.

ORAL SWABS WHICH CONTAIN SPERMATOZOA

A. Dissect swab. Use a clean cutting surface for each different sample.

B. Pipet 150 to 500 µl of PBS into a sterile 1.5 ml microcentrifuge tube. Add swab cutting to tube.

C. Incubate at 4°C for 2 hours or overnight.

D. Twirl the swab with a sterile toothpick for at least 2 minutes to agitate the cells off of the substrate. Remove the swab and toothpick. Store swab or fabric in a sterile tube. (A Costar spin basket may be utilized for the agitation of the cellular component from the substrate. See page 13, E – OPTIONAL)

E. Spin in a microcentrifuge for 2 minutes at 10,000 to 15,000 x g.

F. Without disturbing the pellet, remove all but 50 µl of the supernatant using a microtip disposable transfer pipette and place into the tube containing the swab or fabric. This supernatant can be used for traditional serological analyses if necessary. Re-suspend the pellet in the remaining 50 µl by stirring with a sterile pipette tip.

G. OPTIONAL: remove about 3 µl of the re-suspended sample and spot on a glass microscope slide for examination. Perform Christmas Tree Stain (Serology SOP: Staining of Spermatozoa:Kernechtrot/ Picroindigocarmine).

NOTE: If sperm heads are detected, follow the spermatozoa extraction in the presence of contaminating cells protocol beginning with Step I***. If sperm are not detected, the sample may not be useable.***

BUCCAL SCRAPINGS

A. Scrape the inside of the cheek with a toothpick.

B. Twirl the toothpick directly into 200 µl of 5% Chelex™ in a sterile 1.5 ml microcentrifuge tube.

C. Follow protocol for Inorganic Extraction (Chelex™) Blood Procedure beginning with ***Step F***.

BUCCAL SWAB STANDARDS, STAMPS, ENVELOPE FLAPS, CIGARETTE BUTTS

A. Cut a sample of the item to be analyzed and place into a sterile 1.5 ml microcentrifuge tube. Use a clean cutting surface for each different sample. Pipet 1ml of TE Buffer into the sterile 1.5 ml microcentrifuge tube.

B. Incubate at room temperature for 15 to 30 minutes. Mix occasionally by inversion or gentle vortexing.

C. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

D. Carefully remove supernatant (all but 20 to 30 µl) and discard. Leave the fabric substrate in the tube with the pellet.

E. Add 5% Chelex™ to a final volume of 200 µl.

F. Incubate at 56°C for 15 to 30 minutes or overnight. (Overnight is optimal.)

G. Vortex at high speed for 5 to 10 seconds. Spin in a microcentrifuge for 5 to 10 seconds at 10,000 to 15,000 x g.

H. Incubate in a boiling water bath for 8 minutes.

I. Vortex at high speed for 5 to 10 seconds.

J. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

K. The sample is now ready for the PCR Amplification process.

L. Store the remainder of the supernatant at 2° to 8°C or frozen. To re-use, repeat ***Steps I*** through ***K***.

TISSUES

Tissues should be stored at –20ºC until used.

A. Place a portion of the tissue on a clean surface. Remove a piece of tissue approximately 1 cm^2 and tease apart or cut into small pieces.

B. Add 1 ml TE Buffer. Incubate at room temperature for 15 to 30 minutes. Mix occasionally by inversion or gentle vortexing.

C. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

D. Carefully remove supernatant (all but 20 to 30 µl) and discard. Leave the fabric substrate in the tube with the pellet.

E. Add 5% Chelex™ to a final volume of 200 µl.

F. Incubate at 56°C for 15 to 30 minutes or overnight. (Overnight is optimal.)

G. Vortex at high speed for 5 to 10 seconds. Spin in a microcentrifuge for 5 to 10 seconds at 10,000 to 15,000 x g.

H. Incubate in a boiling water bath for 8 minutes.

I. Vortex at high speed for 5 to 10 seconds.

J. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

K. The sample is now ready for the PCR Amplification process.

L. Store the remainder of the supernatant at 2° to 8°C or frozen. To re-use, repeat ***Steps I*** through ***K***.

HAIR

M. Handling hair with clean forceps, examine the hair under a dissecting microscope for the presence of sheath material. The hair may be placed on a clean piece of white paper. Note possible presence of body fluids on hair.

N. Wash the hair containing sheath material thoroughly to reduce surface dirt and contaminants by immersing the hair in sterile saline. Follow this by either of the following cutting methods.

1. Rinse thoroughly in ethanol and place onto clean paper. Use a clean scalpel or clean scissors to cut a 1 cm portion from the root end of the hair. Because hair may contain cellular material on the surface that may or may not originate from the hair donor, it is advisable to cut off a 1 cm section of the shaft adjacent to the root portion for separate analysis as a control.

   or

2. Place hair into a dish with sterile saline and while still immersed, cut a 1 cm portion from the root end of the hair. It is advisable to cut off a 1 cm section of the shaft adjacent to the root portion as a control.

O. Add the root portion of the hair to 200 µl of 5% Chelex™ in a 1.5 ml microcentrifuge tube. Add 2 µl 10 mg/ml Proteinase K. Make sure that the root portion of the hair is in the Chelex™.

P. Incubate at 56°C (at least 6 to 8 hours) overnight.

Q. Vortex at high speed for 5 to 10 seconds.

R. Spin for 10 to 20 seconds in microcentrifuge at 10,000 to 15,000 x g.

NOTE: Check that the hair root is completely immersed in the Chelex™ solution before boiling.

S. Incubate in a boiling water bath for 8 minutes.

T. Vortex at high speed for 5 to 10 seconds.

U. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g.

V. The sample is now ready for the PCR Amplification Process.

W. Store the remainder of the supernatant at either 2° to 8°C or frozen. To re-use, repeat ***Steps I*** through ***K***.

CONCENTRATION OPTION

Concentration by Microcon™ ultrafiltration may be recommended for Chelex™ extracted samples containing less than 500 ng of DNA. Before discarding any supernatant from the centrifugations, it may be advisable to keep all until the analysis is complete. The aqueous portion of a DNA extract may be concentrated as follows.

A. Assemble a Microcon™ ultrafiltration concentrator unit. To the top of the concentrator, add 100 μl TE Buffer. Transfer the aqueous phase from the DNA extract to the concentrator.

B. Place cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

C. OPTIONAL: Carefully remove the concentrator unit from the assembly and discard the fluid from the filtrate cup. Return the concentrator to the top of the filtrate cup.

D. Add 200 μl TE Buffer to the concentrator. Replace cap and spin in a microcentrifuge at 2500 x g for 10 minutes.

E. Remove the cap and add a measured volume of TE Buffer that is between 20 μl and 200 μl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator into a labeled retentate cup. Discard the filtrate cup.

F. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

G. Discard the concentrator. Cap the retentate cup.

H. If not previously done, estimate the quantity of DNA by slot blot hybridization.

I. After quantification, the sample can be amplified.

J. Store the samples at 4ºC or frozen. Prior to the use of samples after storage, they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

ORGANIC EXTRACTION

All extraction steps must be performed in the DNA extraction/PCR setup laboratory using hoods, reagents, and pipettors dedicated to this area.

WHOLE BLOOD OR BLOODSTAINS

Liquid blood samples should be made into bloodstains. Stains should be air-dried before being stored in individual plastic bags at –20ºC until used.

A. Place the bloodstain (approximately 3 mm by 3 mm) or 3 μl whole blood in a 2.2 ml microcentrifuge tube.

B. To the sample, add 300 μl organic stain extraction buffer, 12 μl 1M DTT and 4 μl of 10 mg/ml Proteinase K solution. Vortex on low speed for 1 second and spin in a microcentrifuge for 2 seconds to force the cutting into the extraction fluid.

C. Incubate the tube at 56°C overnight.

D. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

E. In a fume hood, add 300 μl phenol/chloroform/isoamyl alcohol to the stain extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

F. To a Microcon™ concentrator, add 100 μl TE^{-4}. Transfer the aqueous phase from the tube in ***Step E*** to the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

G. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

H. Add 100 μl TE^{-4} to the concentrator. Replace the cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

I. Remove the cap and add a measured volume of TE^{-4} that is between 20 μl and 200 μl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator into a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 μl for evidentiary (question) samples to 200 μl for reference samples (known) samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples and known samples are generally 100 μl and 200 μl, respectively.

J. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

K. Discard the concentrator. Cap the retentate cup.

L. Estimate the quantity of DNA in the sample by slot blot hybridization.

M. After quantification, the samples can be amplified.

N. Store samples at 2-8ºC or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

VAGINAL SWABS OR SEMEN STAINS

Vaginal swabs or semen stains should be air-dried and stored at –20ºC until used.

A. Using a clean surface, dissect the swab material from the applicator stick or cut a portion of the stained material and place it into a 2.2 ml microcentrifuge tube.

B. To the sample, add 400 µl Tris/EDTA/NaCl, 25 µl 20% Sarkosyl, 75 µl H_2O, and 2 µl of 10 mg/ml Proteinase K. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the material into the extraction fluid.

C. Incubate at 37ºC for 2 hours.

D. Transfer the swab material into a Spin-X basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at maximum speed for 5 minutes.

E. Remove the basket insert from the extract tube. Remove the extracted material from the basket, place in a clean microfuge tube, and freeze if required. Otherwise, discard.

F. While being very careful to not disturb any pelleted material, remove the supernatant fluid from the extract and place it into a new, labeled tube. **THIS SUPERNATANT IS THE NON-SPERM EXTRACTION. ANALYSIS OF THE NON-SPERM FRACTION RESUMES AT** *STEP K***. THE PELLET REMAINING IN THE TUBE IS THE CELL PELLET.**

G. Wash the cell pellet by re-suspending it in 500 µl sperm wash buffer, vortexing the suspension briefly, and spinning the tube in a microcentrifuge at maximum speed for 5 minutes. Remove and discard the supernatant fluid, being careful not to disturb the cell pellet.

H. Repeat ***Step G*** two additional times for a total of three washes of the cell pellet.

NOTE: Additional wash steps are recommended when the ratio of sperm to epithelial cells is low.

I. Re-suspend the pellet by stirring with a sterile pipette tip. Remove about 3 µl of the re-suspended sample and spot on a glass microscope slide for cell examination. Stain with Christmas Tree Stain (refer to the *CBI Forensic Laboratory Serology*

SOP: "Staining of Spermatozoa:Kernechtrot/ Picroindigocarmine"). (***Steps B, C, and D*** spin only, ***F*** remove supernatant, ***G-H*** may be repeated if nucleated or non-nucleated non-sperm cells are detected in the cell examination).

J. To the tube containing the washed pellet, add 150 µl Tris/EDTA/NaCl, 50 µl 20% Sarkosyl, 7 µl 1M DTT, 150 µl H_2O, and 4 µl of 10 mg/ml Proteinase K. Close the tube caps and vortex for 1 second and spin in a microcentrifuge for 2 seconds to force all fluid and material to the bottom of the tubes.

K. Incubate at 56ºC for a minimum of 2 hours.

L. To the tube containing the cell pellet and to the tube containing the female fraction, add 300 µl phenol/chloroform/isoamyl alcohol. Vortex (low speed) both tubes briefly to attain a milky emulsion. Spin the tubes in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions can be performed prior to the purification steps.

M. Assemble a Microcon™ concentrator unit. To the top of the concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step L*** to the top of the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

N. Place a cap on the concentrator unit from the assembly and spin in a microcentrifuge at 2500 x g for 10 minutes.

O. Add 100 µl TE^{-4} to the concentrator. Replace the cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

P. Remove the cap and add a measured volume of TE^{-4} that is between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl to 200 µl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 µl and 200 µl.

Q. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

R. Discard the concentrator. Cap the retentate cup.

S. Estimate the quantity of DNA in the sample by slot blot hybridization.

T. After quantification, the sample can be amplified.

U. Store samples at 4°C or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

SALIVA STAINS OR SWABS

Collect a portion of stain or swab for DNA extraction. Place the material into a 2.2 ml microcentrifuge tube.

A. To the sample, add 300 µl organic stain extraction buffer, 12 µl 1M DTT, and 4 µl 10 mg/ml Proteinase K solution. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the cutting into the extractions fluid.

B. Incubate the tube at 56°C overnight.

C. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

D. In a fume hood, add 300 µl phenol/chloroform/isoamyl alcohol to the stain extract. Vortex the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

E. Assemble a Microcon™ concentrator unit. To the top of the concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step D*** to the top of the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

F. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

G. Add 100 µl TE^{-4} to the concentrator. Replace the cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

H. Remove the cap and add a measured volume of TE^{-4} between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl for evidentiary (question) samples to 200 µl for reference (known) samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples and known samples are generally 100 µl and 200 µl, respectively.

I. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

J. Discard the concentrator. Cap the retentate.

K. Estimate the quantity of DNA in the sample by slot blot hybridization.

L. After quantification, the sample can be amplified.

M. Store samples at 4ºC or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

ENVELOPE FLAPS OR STAMPS

A. Carefully open envelope flap or remove stamps using steam and clean tweezers. Using a sterile cotton swab moistened in sterile, filter-purified water, swab gummed envelope flap or stamp. Cut cotton swab from stick and transfer the cotton to a 2.2 ml microcentrifuge tube. (Optionally, ½ of available dissected material can be placed into the extraction tube.)

B. To the sample, add 300 µl organic stain extraction buffer, 12 µl 1M DTT, and 4 µl 10 mg/ml Proteinase K solution. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the cutting into the extraction fluid.

C. Incubate the tube at 56ºC overnight.

D. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

E. In a fume hood, add 300 µl phenol/chloroform/isoamyl alcohol to the stain extract. Vortex the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

F. Assemble a Microcon™ concentrator unit. To the top of the concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step E*** to the top of the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

G. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

H. Add 100 µl TE^{-4} to the concentrator. Replace the cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

I. Remove the cap and add a measured volume of TE^{-4} that is between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl to 200 µl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 µl and 200 µl.

J. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

K. Discard the concentrator. Cap the retentate cup.

L. Estimate the quantity of DNA in the sample by slot blot hybridization.

M. After quantification, the sample can be amplified.

N. Store samples at 4ºC or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

CIGARETTE BUTTS

A. Collect a portion of the filter and/or paper of the cigarette butt in the area that would have been in contact with the mouth.

B. Cut into smaller pieces and place into a 2.2 ml microcentrifuge tube.

C. To the sample, add 300 µl organic stain extraction buffer, 12 µl 1M DTT, and 4 µl 10 mg/ml Proteinase K solution. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the butt into the extraction fluid.

D. Incubate the tube at 56ºC overnight.

E. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

F. In a fume hood, add 300 µl phenol/chloroform/isoamyl alcohol to the stain extract. Vortex the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

G. Assemble a Microcon™ concentrator unit. To the top of the concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step F*** to the top of the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

H. Place a cap on the concentrator and spin a microcentrifuge at 2500 x g for 10 minutes.

I. Add 100 µl TE^{-4} to the concentrator. Replace the cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

J. Remove the cap and add a measured volume of TE^{-4} that is between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl to 200 µl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 µl and 200 µl.

K. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

L. Discard the concentrator. Cap the retentate cup.

M. Estimate the quantity of DNA in the sample by slot blot hybridization.

N. After quantification, the sample can be amplified.

O. Store samples at 4°C or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

TISSUES

Tissues should be stored at -20°C until used.

A. Place a portion of the tissue on a clean surface. Remove a piece of tissue approximately 1 cm^2.

B. Mince tissue into small pieces and place in a 2.2 ml or 1.5 ml microcentrifuge tube.

C. To the sample, add 300 µl organic stain extraction buffer, 12 µl 1M DTT, and 4 µl 10 mg/ml Proteinase K solution. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the sample into the extraction fluid.

D. Incubate the tube at 56°C overnight.

E. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

F. In a fume hood, add 300 µl phenol/chloroform/isoamyl alcohol to the extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

G. To a Microcon™ concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step F*** to the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

H. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

I. Add 100 µl TE^{-4} to the concentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

J. Remove the cap and add a measured volume of TE^{-4} that is between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl to 200 µl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 µl and 200 µl.

K. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

L. Discard the concentrator. Cap the retentate cup.

M. Estimate the quantity of DNA in the sample by slot blot hybridization.

N. After quantification, the sample can be amplified.

O. Store samples at 4°C or frozen. Prior to the use of samples after storage they should be vortexed briefly and spin in a microcentrifuge for 5 seconds.

HAIRS

UNMOUNTED HAIR SPECIMENS

A. Handling hair with clean forceps, examine the hair under a dissecting microscope for the presence of sheath material. The hair may be placed on a clean piece of white paper. Note possible presence of body fluids on hair.

B. Wash the hair containing sheath material thoroughly to reduce surface dirt and contaminants by immersing the hair in sterile saline. Follow this by either of the following cutting methods.

1. Rinse thoroughly in ethanol and place onto clean paper. Use a clean scalpel or clean scissors to cut a 1 cm portion from the root end of the hair. Because hair may contain cellular material on the surface that may or may not originate from the hair donor, it is advisable to cut off a 1 cm section of the shaft adjacent to the root portion for separate analysis as a control. Alternatively, the entire hair can be placed into the tube.

   or

2. Place hair into a dish with sterile saline and while still immersed, cut a 1 cm portion from the root end of the hair. It is advisable to cut off a 1 cm section of the shaft adjacent to the root portion as a control.

C. To the sample, add 300 µl organic stain extraction buffer, 12 µl 1M DTT, and 4 µl 10 mg/ml Proteinase K solution. Vortex on low speed for 1 second and spin in a microcentrifuge for 2 seconds to force the sample into the extraction fluid.

D. Incubate the tube at 56°C overnight.

E. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

F. In a fume hood, add 300 µl phenol/chloroform/isoamyl alcohol to the extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

G. To a Microcon™ concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step F*** to the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

H. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

I. Add 100 µl TE^{-4} to the concentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

J. Remove the cap and add a measured volume of TE^{-4} that is between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl to 200 µl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 µl and 200 µl.

K. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

L. Discard the concentrator. Cap the retentate cup.

M. Estimate the quantity of DNA in the sample by slot blot hybridization.

N. After quantification, the sample can be amplified.

O. Store samples at 4°C or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

SLIDE-MOUNTED HAIR SPECIMENS

A. Loosen the slide coverslip by carefully pipetting xylene around the coverslip edges. If the coverslip does not loosen, the entire slide can be placed into a Petri dish and covered with xylene for one or more hours until the coverslip has loosened.

B. After removal of the coverslip, remove the hair and rinse it thoroughly with xylene.

C. Continue processing at ***Step B*** of the procedure for Unmounted Hair Specimens.

BONE

Bone specimens should be store at –20ºC until processed.

A. Cut the bone specimen to a size of approximately 2 cm x 5 cm. (Best results are obtained if the bone is pulverized in a sterile fashion).

B. Transfer the bone specimen to a 2.2 ml or 1.5 ml microcentrifuge tube.

C. To the sample, add 300 µl organic stain extraction buffer, 12 µl 1M DTT, and 4 µl 10 mg/ml Proteinase K solution. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the sample into the extraction fluid.

D. Incubate the tube at 56°C overnight.

E. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

F. In a fume hood, add 300 µl phenol/chloroform/isoamyl alcohol to the extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

G. To a Microcon™ concentrator, add 100 µl TE^{-4}. Transfer the aqueous phase from the tube in ***Step F*** to the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

H. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

I. Add 100 µl TE^{-4} to the concentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

J. Remove the cap and add a measured volume of TE^{-4} that is between 20 µl and 200 µl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 µl to 200 µl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 µl and 200 µl.

K. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

L. Discard the concentrator. Cap the retentate cup.

M. Estimate the quantity of DNA in the sample by slot blot hybridization.

N. After quantification, the sample can be amplified.

O. Store samples at 4ºC or frozen. Prior to the use of samples after storage they should be vortexed briefly and spun in a microcentrifuge for 5 seconds.

TEETH

In general, for DNA analysis, large teeth with no restorations should be chosen over smaller, restored teeth. Thus a non-restored, molar tooth is the tooth of choice for DNA recovery.

A. Clean the outer surface of the tooth with alcohol.

B. Split or crush tooth utilizing sterile instruments. Extirpate pulp tissue.

C. Place the processed tooth tissue into a microcentrifuge tube. (If pulp tissue is limited in quantity, a portion of the crushed dentate may be placed in the microcentrifuge tube). To the sample add 300 μl organic stain extraction buffer, 12 μl 1M DTT, and 4 μl 10 mg/ml Proteinase K solution. Close the tube cap.

D. Vortex for 1 second and spin in a microcentrifuge for 2 seconds to force the sample into the extraction fluid.

E. Incubate the tube at 56°C overnight.

F. Spin in a microcentrifuge for 2 seconds to force condensation into the bottom of the tube.

G. In a fume hood, add 300 μl phenol/chloroform/isoamyl alcohol to the extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

Note: If necessary, additional organic extractions may be performed prior to the purification steps.

H. To a Microcon™ concentrator, add 100 μl TE^{-4}. Transfer the aqueous phase from the tube in ***Step G*** to the concentrator. Avoid pipetting organic solvent from the tube into the concentrator.

I. Place a cap on the concentrator and spin in a microcentrifuge at 2500 x g for 10 minutes.

J. Add 100 μl TE^{-4} to the concentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 2500 x g for 10 minutes.

K. Remove the cap and add a measured volume of TE^{-4} that is between 20 μl and 200 μl to the concentrator. Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

Note: Final recovery volumes following purification generally range from 20 μl to 200 μl for evidentiary samples. The final recovery volumes for the extraction reagent blanks for evidentiary samples are generally between 20 μl and 200 μl.

L. Spin the assembly in a microcentrifuge at 2500 x g for 5 minutes.

M. Discard the concentrator. Cap the retentate cup.

N. Estimate the quantity of DNA in the sample by slot blot hybridization.

O. After quantification, the sample can be amplified.

P. Store samples at 4°C or frozen. Prior to the use of samples after storage, they should be vortexed briefly and spun in microcentrifuge for 5 seconds.

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BLOOD EXTRACTION USING FTA CARDS

FTA cards are chemically treated to tightly bind DNA. Blood from database samples is spotted onto the cards. Punches from the cards are subjected to a series of washes that remove cellular components. The DNA remains bound to the cards and never enters solution, so precautions concerning having multiple tube lids open simultaneously and changing pipette tips between samples are relaxed for this extraction procedure. In addition, since a large amount of input DNA is bound to the punch, the amplification cycle number may be reduced.

A. Using a clean 1.2 mm Harris punch, remove a sample from the stain and place it into an amplification tube.

B. Add 200 µl FTA Purification Reagent to each tube. Cap each tube and vortex 1 to 2 seconds at slow speed.

C. Allow the tubes to sit for 5 minutes at room temperature with a second brief vortex halfway through the incubation.

D. After the 5 minute incubation, vortex for a third time and then carefully remove as much of the reagent as possible.

E. Repeat ***Steps C*** *and* ***D*** an additional two times for a total of three washes with the FTA Purification Reagent.

F. After the FTA Purification Reagent has been removed for the third time, add 200 µl TE (10mM Tris-HCl, pH 8.0, 0.1mM EDTA). Cap each tube and vortex 1 to 2 seconds at low speed.

G. Allow the tubes to sit for 5 minutes at room temperature with a brief vortex halfway through the incubation.

H. Remove the TE and replace with an additional 200 µl TE. Cap each tube and vortex 1 to 2 seconds at low speed.

I. Allow the tubes to sit for 5 minutes at room temperature with a brief vortex halfway through the incubation.

J. Remove the TE and allow the FTA paper punch to air dry. Alternatively, go directly to ***Step K***.

K. Add TE to the tube containing the washed paper punch sample; then add PCR amplification mix directly to the punch containing the purified immobilized DNA.

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QUANTIBLOT®

A. Reagent Preparation

1. Preparation of reagents supplied

   Chromogen:TMB Solution. Bring the Chromogen:TMB (TMB) to room temperature (15° to 30°C). Before opening the bottle, tap it on the lab bench to shake the TMB to the bottom of the bottle. Remove the stopper carefully to prevent loss of the powder. Slowly add 30 ml of room temperature reagent grade 100% ethanol to the bottle.

   Do NOT use ethanol that has been stored in a metal container. Do NOT use 95% ethanol or other alcohols.

   Recap the bottle. Seal the stopper with Parafilm®. Shake in an upright position on an orbital shaker for 30 minutes or until completely dissolved. Store in bottle at 2° to 8°C and protect from rust. Under these conditions the Chromogen Solution is stable for six months after preparation.

2. Human DNA Standards. Prepare a two-fold serial dilution of the DNA Standard A (provided in Kit) in TE Buffer as follows.

   a. Label seven 0.5 ml DNA/RNA free microcentrifuge tubes A through G.

   b. Vortex the DNA Standard A to mix it thoroughly.

   c. Transfer 120 µl DNA Standard A into the tube labeled A.

   d. Aliquot 60 µl TE Buffer into each of the six remaining tubes labeled B through G.

   e. Add 60 µl DNA Standard A (tube A) to the 60 µl TE Buffer in tube B. ***Vortex to mix*** thoroughly.

   f. Add 60 µl diluted DNA Standard B (tube B) to the 60 µl TE Buffer in tube C. ***Vortex to mix*** thoroughly.

   g. Add 60 µl diluted DNA Standard C (tube C) to the 60 µl TE Buffer in tube D. ***Vortex to mix*** thoroughly**.**

   h. Continue the serial dilution through tube G.

   i. If the dilution steps are performed as described in ***Section 2, c - h*** above, the seven DNA Standard tubes (tubes A through G) will have the concentrations of human DNA listed in ***Table 1***.

Table 1 — DNA Standards and Concentrations

DNA Standard	Concentration (ng/μl)	Quantity DNA per 5 μl (ng)
A	2	10
B	1	5
C	0.5	2.5
D	0.25	1.25
E	0.125	0.625
F	0.0625	0.3125
G	0.03125	0.15625

NOTE: Store the diluted DNA Standards at 2° to 8ºC. The DNA Standards A through G are stable for at least three months at 2° to 8ºC.

B. Information Regarding Protocols

This entire section should be read before slot blotting/immobilization of DNA.

The QuantiBlot® Human DNA Quantitation Kit contains reagents for at least 10 hybridization reactions. Each hybridization reaction should include the following ten control samples: seven DNA Standards, the two DNA Calibrators and one blank [Spotting Solution only]. An additional 38 samples can be spotted on the membrane, for a total of up to 48 samples per hybridization reaction. DNA Calibrators are provided as an internal control for DNA Standard performance.

C. Slot Blotting/Immobilization of DNA

NOTE: Wear clean disposable laboratory gloves while preparing samples. Follow safety recommendations provided by manufacturer for handling chemicals. Comply with any and all laws, regulations, or orders with respect to the disposal of any hazardous or toxic chemical, material, substance, or waste.

Before beginning this section, assemble the required reagents, supplies, and equipment as follows.

DNA Standards (A through G)
DNA Calibrator 1 (provided in Kit)
DNA Calibrator 2 (provided in Kit)
Slot Blot Apparatus
Spotting Solution
Pre-Wetting Solution
Biodyne® B nylon membrane

1. Determine the number of samples to be analyzed including the seven Human DNA Standards (A through G), the DNA Calibrators 1 and 2 (provided in Kit), and the one blank (Spotting Solution only). Aliquot 150 µl Spotting Solution into a new 0.5 ml GeneAmp PCR Reaction Tube for each sample.
2. Label seven of the tubes containing 150 µl Spotting Solution as follows: A, B, C, D, E, F, and G. Label two other tubes containing 150 µl Spotting Solution as DNA Calibrator 1 and DNA Calibrator 2.
3. Vortex the seven DNA standards and the two DNA Calibrators. Add 5 µl of each solution to the corresponding labeled tube containing 150 µl Spotting Solution.

NOTE: Sample DNA should be $MgCl_2$-free, See** Troubleshooting **Section.

4. Add 1 to 5 µl of each test sample DNA to the remaining tubes containing 150 µl Spotting Solution.
5. While wearing clean gloves, cut a piece of Biodyne® B membrane to 11.0 cm x 7.9 cm. Cut a small notch in the upper right corner of the membrane to mark orientation. Place the membrane in the Hybridization Tray (***Part No. N808-0136***) containing 50 ml Pre-Wetting Solution. Incubate at room temperature for 1 to 30 minutes.

NOTE: The following protocol is for use with GIBCO BRL, the Convertible® slot blot apparatus. Refer to GIBCO BRL instructions for additional details.

6. Using forceps, remove the membrane from the Pre-Wetting Solution. Place the membrane on the gasket of the slot blot apparatus, then place the top plate of the slot blot apparatus on top of the membrane.

   Turn on the vacuum source (i.e., turn on house vacuum line or vacuum pump). ***Turn off the sample vacuum and turn on the clamp vacuum*** on the slot blot apparatus. Push down on the top plate to insure the formation of a tight seal. Pour off the Pre-Wetting Solution and rinse the Hybridization Tray thoroughly with DI H_2O.

7. Use a new pipette tip for each sample. Pipet each sample (approximately 155 µl) into a different well of the slot blot apparatus. Slowly dispense each sample directly into the center of each well of the slot blot apparatus ensuring that the pipette tip is approximately 5mm above the membrane.

8. After all samples have been pipetted into the wells of the slot blot apparatus, ***slowly turn on the sample vacuum*.** Leave the sample vacuum on until all of the samples have been drawn through the membrane (approximately 30 seconds). Inspect each slot that contains a sample for a uniform blue band. (If a uniform blue band is not visible, refer to ***Section T***.) ***Turn off the sample vacuum****.*

9. ***Turn off the clamp vacuum. Turn off the vacuum source*.** Disassemble the slot blot apparatus and remove the membrane.

Proceed to *Section D* ***immediately. Do NOT allow the membrane to dry-out.***

NOTE: After each use, soak the slot blot apparatus in a large volume of 0.1% SDS solution (approximately 5 to 15 minutes). Using a disposable lab towel, clean the gasket and the side of the top plate that contacts the membrane. Then rinse the slot blot apparatus with an excess of water and allow to dry at room temperature. Never use bleach.

D. DNA Hybridization

The following section involves the hybridization of biotinylated QuantiBlot® D17Z1 Probe to DNA samples immobilized on the nylon membrane, the binding of Enzyme Conjugate:HRP-SA to the hybridized probe, and a stringent wash to remove non-specifically bound probe.

Before starting the DNA Hybridization procedure, assemble the required reagents and equipment as follows.

QuantiBlot® D17Z1 Probe (provided in Kit)
Enzyme Conjugate:HRP-SA (provided in Kit)
Hybridization Solution
QuantiBlot® Wash Solution
Citrate Buffer
30% Hydrogen Peroxide
Hybridization Tray and Lid
Hybridization Tray Retainer

Do NOT allow the membrane to dry at any point in the protocol. Minimize the time the membrane is not submerged in solution. Use the Hybridization Tray with lid for all steps.

Warm the Hybridization Solution and the QuantiBlot® Wash Solution to between 37° and 50°C in either a water bath or an incubator. ***All solids must be in solution before use.*** Mix well.

NOTE: Clean, disposable gloves should be worn throughout the DNA Hybridization **(**Section D**)** ***and Detection Steps*** **(**Section E**).**

1. Pre-hybridization: transfer the membrane to 100 ml pre-warmed Hybridization Solution in the Hybridization Tray. Add 5 ml 30% H_2O_2. Place the lid on the tray. Use the Hybridization Tray Retainer or a lead weight to keep tray from floating in the water bath. Rotate in a 50°C (± 1°C) water bath (50 to 60 rpm) for 15 minutes (± 2 minutes). Pour off the solution.
2. Hybridization: add 30 ml Hybridization Solution to the Hybridization Tray containing the membrane. Tilt the tray to one side and add 20 µl QuantiBlot® D17Z1 Probe to the Hybridization Solution. Place the lid on the tray. Rotate in a 50°C (± 1ºC) water bath (50 to 60 rpm) for 20 minutes (± 2 minutes). Pour off the solution.
3. Rinse the membrane briefly in 100 ml pre-warmed QuantiBlot® Wash Solution by rocking the tray for several seconds. Pour off the solution.
4. Stringent Wash/Conjugation: add 30 ml pre-warmed QuantiBlot® Wash Solution to the Hybridization Tray. Tilt the tray to one side and add 180 µl Enzyme Conjugate:HRP-SA to the 30 ml Wash Solution. Place the lid on the tray. Rotate in a 50°C (± 1°) water bath (50 to 60 rpm) for 10 minutes (± 1 minute). Pour off the solution.
5. Rinse the membrane thoroughly for 1 minute in 100 ml pre-warmed QuantiBlot® Wash Solution by rocking the tray or rotating it on an orbital shaker (100 to 125 rpm) at room temperature. Pour off the solution. Rinse again for 1 minute. Pour off the solution.

6. Wash the membrane by adding 100 ml pre-warmed QuantiBlot® Wash Solution to the tray. Place the lid on the tray. Rotate at room temperature on an orbital shaker (100 to 125 rpm) at room temperature for 15 minutes. Pour off the solution.

7. Rinse the membrane briefly in 100 ml Citrate Buffer by rocking the tray. Pour off the solution.

E. Colorimetric Detection

1. Prepare the Color Development Solution ***not more than 10 minutes before use***. Add the following reagents in the order listed to a glass flask and mix thoroughly by swirling. ***Do NOT vortex***.

   To 30 ml Citrate Buffer add 1.5 ml Chromogen:TMB Solution and 30 µl 3% H_2O_2.

2. Add Color Development Solution to the membrane in the tray. ***Cover the tray with the lid to protect the membrane from strong light***.

3. Shake at room temperature on an orbital shaker (50 to 60 rpm) for 20 to 30 minutes until all of the DNA Standards are visible.

4. Remove tray from shaker, pour off liquid.

5. Stop the color development by washing in deionized H_2O (100 ml). Shake for 5 to 10 minutes (50 to 60 rpm) with the lid on the tray. Repeat for a total of three washes.

6. Photograph the membrane when it is wet. Saran Wrap® may be placed over the membrane during photography to prevent it from drying out.

   a. Place the wet membrane on a flat non-absorbent surface. Keep the membrane wet throughout the photographic procedure. Minimize exposure to strong light.

   b. Use a Polaroid camera with Polapan 400 or Type 52 (black and white) film or Type 59 or 559 (color) film, or other photographic recording device.

   c. Follow exposure and development instructions.

   NOTE: For black and white photography, an orange filter (Wratten 22 or 23A) will enhance contrast.

   d. Following photography, the membrane may be air-dried on any hard non-absorbent surface. Protect from light and oxidizing agents (e.g., acid treated paper, bleach, and nitric acid). The blue color on the membrane will fade upon drying.

F. Results Interpretation

Results are interpreted by comparing the signal intensity of the DNA test sample to the signal intensity obtained for the DNA Standards. The signal intensity for a sample reflects the total amount of DNA spotted on the membrane. The seven DNA Standards represent the following quantities of DNA spotted on the membrane: 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 ng **(*See Table 1*)**.

The DNA Calibrators are used to provide DNA of a known concentration to verify that the DNA Standards were correctly diluted and are providing correct results for the test samples. For example, the DNA Calibrator 1 has a stock concentration of 0.7 ng/μl. Five μl of this control was added to 150 μl of Spotting Solution and the entire 155 μl was spotted on the membrane. Thus, 3.5 ng of this sample was spotted on the membrane (0.7 ng/μl · 5 μl = 3.5 ng). The signal obtained for this control sample should have an intensity that is between the 2.5 and 5 ng DNA Standards. Likewise, the DNA Calibrator 2 should have an intensity that is between the 0.3125 and 0.625 ng DNA Standards. If not, see the ***Troubleshooting Section.***

The concentration of a DNA test sample is determined as follows.

1. Determine the quantity of DNA test sample spotted on the membrane by comparing its signal intensity to the intensity of the DNA Standards.
2. Divide this quantity by the volume of DNA test sample added to the Spotting Solution (typically 5 μl DNA test sample is added to 150 μl Spotting Solution).

   This calculation gives DNA concentration in ng/μl.

PERFORMANCE CHARACTERISTICS

The QuantiBlot® Human DNA Quantitation Kit will be able to detect and quantitate 0.15 to 10 ng of human DNA per test sample.

TROUBLESHOOTING

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
1. No signal or low sensitivity (0.15 ng DNA Standard not visible).	Use of a membrane other than Biodyne® B.	Use Biodyne B® nylon membrane. Do not use membranes that have a neutral charge.
	Incorrect NaOH or EDTA concentrations in Spotting Solution.	Prepare Spotting Solution correctly.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	Water bath temperature too high.	Water bath temperature should be 50°C (± 1°C).
	DNA Probe was not added at hybridization step.	Add QuantiBlot® D17Z1 Probe.
	Enzyme conjugate was not added.	Add Enzyme Conjugate: HRP-SA at indicated step in protocol. Use 180 µl Enzyme Conjugate:HRP-SA for colorimetric (TMB) detection.
	Hydrogen peroxide was inactive.	Prepare a new Color Development Solution using a fresh bottle of hydrogen peroxide.
	Presence of $MgCl_2$ in the DNA sample.	Concentrations of $MgCl_2$ >0.3mM can result in reduced sensitivity. Prepare all DNA dilutions in TE Buffer. Any $MgCl_2$ can be removed from samples by microdialysis using Centricon® 100 spin units (follow manufacturer's directions).
2. Areas of low sensitivity across the membrane.	Membrane slipped up onto the side of the Hybridization Tray during Hybridization or Stringent Wash steps.	Reduce the rotation rate of the water bath to 50-60 rpm. Check that the membrane is fully submerged in the bottom of the Hybridization Tray before shaking.
	Membrane dried-out significantly at some point in the protocol.	Do not allow the membrane to dry at any point in the protocol.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
3. Non-uniform signal intensity within a slot.	Bubble(s) in slot blot wells when sample was pipetted into well, or when vacuum was applied.	Slowly pipet the Spotting Solution directly over the center of the wells of the slot blot apparatus, with the pipette tip raised approximately 5mm above the membrane. Turn on the sample vacuum slowly, not all at once. After being drawn through the membrane, the sample should appear as a uniform blue band on the membrane. If the entire sample is not drawn through the membrane, turn off the sample vacuum. Pipet the sample back into the pipette tip; then pipet the sample back into the well of the slot blot apparatus. Turn on the sample vacuum to draw the sample through the membrane.
4. Filter background.	No or low SDS in the Hybridization Solution or in the QuantiBlot® Wash Solution.	Prepare solutions with proper concentrations of SDS.
	Membrane was not pre-wetted prior to slot blotting.	Pre-wet the membrane in Pre-Wetting Solution prior to slot blotting.
	Too much Enzyme Conjugate:HRP-SA was added.	Use 180 µl Enzyme Conjugate:HRP-SA for colorimetric detection.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	Lack of thorough rinsing at ***Section D.5*** of the DNA Hybridization protocol.	Thoroughly rinse twice, for 1 minute each, using 100 ml pre-warmed Wash Solution at this step. These two rinse times can be extended beyond 1 minute if necessary.
	Slot blot apparatus not cleaned thoroughly after last use.	Immediately after each use, soak the slot blot apparatus in a large volume of 0.1% SDS solution. Never use bleach.
5. The DNA Calibrators do not quantitate correctly with respect to the DNA Standards.	DNA Standard serial dilutions prepared incorrectly.	Prepare two-fold serial dilutions of DNA Standard A in TE Buffer as described. Add 5 μl of each dilution to 150 μl Spotting Solution for slot blotting.
6. Signals obtained for non-human DNA samples.*	Water bath temperature too low.	Water bath temperature should be 50° (C ± 1ºC).
	SSPE concentration too high in QuantiBlot® Wash Solution.	Check that the 20X SSPE solution and the QuantiBlot® Wash Solution were prepared correctly.

*DNA from primate species may give signals similar to those obtained from equivalent amounts of human DNA. In Roche Molecular Systems (RMS) laboratories, 30 ng to 300 ng quantities of non-primate DNA samples result in either no signals or signals that are less than or equal to the signal obtained for 0.15 ng of human DNA. The following non-primate DNA samples have been tested in RMS laboratories: E. coli, yeast, dog, cat, mouse, rat, pig, cow, chicken, fish and turkey.

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AMPLITYPE® PM + DQA1

STORAGE AND STABILITY

A. Store the AmpliType® PM + DQA1 PCR Amplification and Typing Kit components at 2° to 8ºC. Isolate the kit from any sources of contaminating DNA, especially amplified PCR product.

B. Store the AmpliType® DNA Probe Strips protected from light at 2° to 8ºC. Store the DNA Probe Strips with the desiccant in the glass tube and insure that the screw-cap is securely tightened.

C. Under these conditions, components of the kit are stable through the control date printed on the label.

D. The Chromogen Solution is stable for 6 months after its preparation when stored at 2° to 8°C.

PREPARATION OF REAGENTS SUPPLIED

A. Chromogen:TMB Solution. Bring the bottle of Chromogen:TMB (TMB) to room temperature (15° to 30ºC). Before opening the bottle, tap it on the lab bench to shake the TMB to the bottom of the bottle. Remove the stopper carefully to prevent loss of the powder. Slowly add 30 ml room temperature, reagent grade 100% ethanol to the bottle.

Do NOT use ethanol that has been stored in a metal container. Do not use 95% ethanol or other alcohols.

Recap the bottle. Seal the stopper with Parafilm®. Shake in an upright position on an orbital shaker for 2 hours or until completely dissolved. Store bottle at 2° to 8°C.

Under these conditions the Chromogen Solution is stable for six months after preparation.

B. AmpliType® PM + DQA1 PCR Reaction Mix. Upon first use of the AmpliType® PM + DQA1 PCR Amplification and Typing Kit, remove the bottle of AmpliType® PM + DQA1 PCR Reaction Mix and carefully aliquot 40 µl into autoclaved tubes (GeneAmp® Autoclave Thin-Walled Reaction Tubes for the DNA Thermal Cycler 480) using a dedicated positive displacement repipettor or a pipettor with hydrophobic filter plugged tips.

CAUTION: This step must be performed either in a biological hood or in a room free from amplified DNA.

Pipet 2 μl of Bovine Serum Albumin (Fraction 5:8 μg/μl) into each tube using a sterile pipette. Insure that the tubes are capped tightly. Place PCR Reaction Mix tubes in a rack not used for DNA preparation or amplified DNA handling. Store tubes separated from any source of DNA at 2° to 8ºC.

NOTE: All of the AmpliType® PM + DQA1 PCR Reaction Mix should be aliquoted at the same time to decrease the possibility of contamination through repeated opening of the AmpliType® PM + DQA1 PCR Reaction Mix bottle.

Sections Precautions ***and*** PCR Amplification Protocols ***should be read before preparing AmpliType® PM + DQA1 PCR amplification reactions.***

PRECAUTIONS

The sensitivity of PCR allows minute quantities of DNA to be typed using the AmpliType® PCR Amplification and Typing Kits. Contamination of the samples by handling or by exposure to any other source of human DNA is an important concern. Precautions should be taken to prevent the following three types of contamination: 1) contamination with exogenous human genomic DNA; 2) cross-contamination between extracted DNA samples; 3) "carryover" of PCR product from one amplification to the next.[18, 28] At a minimum, the pre-PCR amplification area must be separated from the post-PCR amplification area.

PCR AMPLIFICATION PROTOCOLS

The following protocols detail the PCR amplification and typing procedures specific for the AmpliType® PCR Amplification and Typing Kits.

A dedicated area, such as a biological hood or a separate room, should be used for preparing AmpliType® PCR amplification reactions. All equipment and supplies used to prepare amplifications should be kept in this dedicated "clean" area at all times. Do not use these items to handle amplified DNA or other potential sources of contaminating DNA. Trace amounts of amplified DNA, if carried over into other samples before amplification, can lead to results that are not interpretable. ***Do NOT bring amplified DNA, or equipment and supplies used to handle amplified DNA, into the DNA extraction/PCR setup area.***

NOTE: Wear clean disposable laboratory gloves while preparing samples for PCR amplification. Change gloves frequently or whenever there is a chance they have been contaminated with DNA.

A. Turn on the GeneAmp® PCR Instrument System (Thermal Cycler 480) and program as follows (see ***Instrument Manual***).

File # 30:	32 Cycles	
Melt:	1 minute	94ºC
Anneal:	30 seconds	60ºC
Extend:	30 seconds	72ºC
File # 10:	Final Step / 7 minutes	72ºC
File # 20:	Soak indefinite	15ºC

NOTE: These programs may be saved as User Files for later use (see Instrument Manual).

As with any calibrated piece of laboratory equipment, the GeneAmp® PCR Instrument System should have a documented temperature verification test performed semiannually.

B. Prepare the DNA test samples for addition to the PCR amplification reactions. Each DNA sample shall be quantitated with the QuantiBlot® Human DNA Quantitation Kit (Part No. N808-0114). The final DNA concentration should be in the range of 0.1 to 0.5 ng/μl so that 2 to 10 ng of DNA will be added to the PCR reaction in a volume of 18 μl. If the sample DNA concentration is greater than 0.5 ng/μl dilute a portion of it with autoclaved DI H_2O. For most samples, 2 to 5 ng is sufficient. If the DNA sample contains degraded DNA, it may be appropriate to add >10 ng of DNA.

C. Determine the number of samples to be amplified, including positive and negative controls. The Control DNA 1 provided in the kit shall be amplified each time the kit is used. A negative control consisting of 40 μl of AmpliType® PM + DQA1 PCR Reaction Mix, 40 μl of AmpliType® PM +DQA1 Primer Set, 2 μl of Bovine Serum Albumin, and 18 μl of autoclaved DI H_2O shall also be included with each set of amplification reactions.

D. Transfer the PCR amplification reagents to the designated clean area. Place the required number of reaction tubes containing 40 μl of aliquoted AmpliType® PM + DQA1 PCR Reaction Mix and 2 μl of Bovine Serum Albumin in a rack not used for the preparation of DNA or the handling of amplified DNA. Label the reaction tubes.

E. Insure that the solution is at the bottom of each tube by spinning the tubes briefly in a microcentrifuge. Open the caps with a clean microcentrifuge tube de-capping tool (this de-capping tool should not have been used on tubes containing amplified or extracted DNA). ***Avoid touching the inside surface of the tube caps.***

F. Pipet 40 μl of the AmpliType® PM +DQA1 Primer Set (provided in the kit) into each tube, including control tubes, using a sterile dedicated positive displacement pipette

or a pipettor with tips plugged with hydrophobic filters. Pipet carefully and at a slight angle to minimize mixing and to avoid splashing the solution.

It is important to begin the cycling process within 20 minutes after the addition of the AmpliType® Primer Set to the AmpliType® PCR Reaction Mix to minimize the formation of primer dimer[29] and other non-specific PCR products.

G. Carefully add 2 drops of mineral oil from the dropper bottle provided in the kit to all tubes, including the controls, before proceeding.

Be careful NOT to touch the reaction tubes with the dropper bottle.

Cap each tube loosely.

Do NOT vortex, mix or spin.

NOTE: Each AmpliType® PM + DQA1 PCR amplification is performed in a final volume of 100 μl. Exactly 18 μl has been allocated for sample addition.

H. For each of the following additions, complete the processing of each tube before proceeding to the next tube. No more than one tube should be opened at a time when making the following additions. Use a new sterile pipette tip for each addition. Open the tube and carefully add 18 μl of extracted Sample or Control DNA 1. Carefully insert the pipette tip through the mineral oil layer. Discard the pipette tip and re-cap the tube tightly before proceeding to the next sample. Do NOT vortex, mix or spin.

Prepare tubes as follows.

1. DNA TEST SAMPLE TUBES: add 18 μl of sample DNA to each labeled DNA test sample tube. If needed, dilute a portion of the sample DNA with autoclaved DI H_2O so that only 2 ng to 10 ng of DNA will be added to the PCR reaction in a volume of 18 μl.
2. POSITIVE CONTROL TUBE: vortex the Control DNA 1 and spin the tube briefly in a microcentrifuge before use to remove any liquid from the cap. Add 18 μl of the 100 ng/ml (1.8 ng) Control DNA 1 to the labeled positive control tube.
3. NEGATIVE CONTROL TUBE: add 18 μl of autoclaved DI H_2O to the labeled negative control tube.

I. As soon as all samples have been added, place the tubes into the GeneAmp® PCR Instrument System.

Push the tubes down completely into the block.

The position of each tube in the block should be recorded.

J. Start the 32-cycle amplification file. Verify the cycling parameters by monitoring the first cycle. Check the tubes after the first cycle to insure they are all still seated tightly in the block.

PREPARATION OF PCR PRODUCT FOR DETECTION

After the PCR amplification process, remove the tubes from the GeneAmp® PCR Instrument System. Prior to DNA hybridization **(Section *DNA Hybridization*)**, open the tubes one at a time and add **5 µl 200mM disodium EDTA.** Use a new pipette tip for each addition. Carefully insert the pipette tip through the mineral oil layer. Discard the pipette tip and recap the tube before proceeding to the next tube.

NOTE: EDTA must be added prior to heat denaturation of the samples.

Samples are now ready for DNA hybridization and color development.

Amplified samples containing 9.5mM EDTA may be stored at 2° to 8°C for 2 months or at -20°C for 6 months.

The continued acceptable performance of these samples beyond these periods may vary with the sample.

Store amplified DNA samples separate from all PCR amplification reagents, extracted DNA samples, and casework samples.

DNA HYBRIDIZATION

The AmpliType® PM +DQA1 DNA Hybridization process involves 3 steps performed sequentially as follows: 1) hybridization of amplified DNA to DNA probe strips, 2) binding of HRP-SA to hybridized PCR products, and 3) stringent wash to remove non-specifically bound PCR products. Color Development follows the stringent wash step.

Certain steps in the following procedures involve the aspiration of solutions containing amplified DNA.

Before starting the DNA Hybridization and Color Development procedures, assemble the required reagents and equipment as follows.

Enzyme Conjugate:HRP-SA (included in kit)
Chromogen:TMB Solution (included in kit; prepared in ***Section A***)
Hybridization Solution
PM + DQA1 Wash Solution
Citrate Buffer
3% Hydrogen Peroxide
AmpliType® PM + DQA1 DNA Probe Strips (Included in Kit)
AmpliType® DNA Typing Trays (***Part No. N808-0065***)

Clean, disposable gloves should be worn throughout the DNA Hybridization and Color Development steps to avoid soiling the DNA probe strips and to protect fingers from the 95°C block. Gloves and lab coats should be discarded when leaving the work area to avoid transport of amplified DNA from the work area.

A. Heat the rotating water bath to 55°C and maintain the temperature between 54°C and 56ºC. It is essential to check the temperature with a calibrated complete immersion thermometer before the hybridization step is performed.

B. The water level should be ¼ inch above the shaker platform. The water level should not be higher than ¼ inch since higher levels may result in water splashing into the wells of the tray. An empty tray can be used to test the water level prior to use. A rotating water bath set at 50 to 90 rpm is necessary for the hybridization and wash steps. Maintain the rotation and the temperature of the water bath throughout ***Section I***. Do NOT use a hot air shaker.

C. Heat an incubator to 50° to 55°C and warm the Hybridization Solution and the PM + DQA1 Wash Solution to 50° to 55°C. All solids must be completely dissolved and all solutions should be well mixed before use.

D. Using filter forceps, remove the required number of AmpliType® DNA Probe Strips from the tube. Using the pen included with the AmpliType® DNA Typing Trays, label each strip in the space at the right edge of the strip. The use of other pens is not recommended because some inks may affect the quality of the typing results. Place one DNA probe strip in each clean well of the AmpliType® DNA Typing Tray. Strips should all be in the same orientation.

NOTE: AmpliType® PM and AmpliType® HLA DQA1 DNA Probe Strips can be used to type PCR products from the same PM + DQA1 amplification reaction at the same time but the DNA probe strips must be placed in separate wells of the tray.

E. Prepare the GeneAmp® PCR Instrument System to denature the amplified DNA by setting the temperature parameter to 95°C (***see Instrument Manual***). Start the program.

F. Place the tubes in the GeneAmp® PCR Instrument System after it reaches 95°C. Press the tubes down tightly in the block. Denature the amplified DNA by incubation at 95°C for 3 to 10 minutes. Keep each tube at 95°C until use.

G. As shown in ***Figure 1***, tilt the DNA Typing Tray towards the labeled end of the strips. The DNA Typing Tray Lid turned upside down may be used as the solid support. Add 3 ml pre-warmed Hybridization Solution to each well at the labeled end of each strip.

Do not wet the remainder of the strip.

NOTE: Hybridization solution solids must be completely dissolved before adding to the tray.

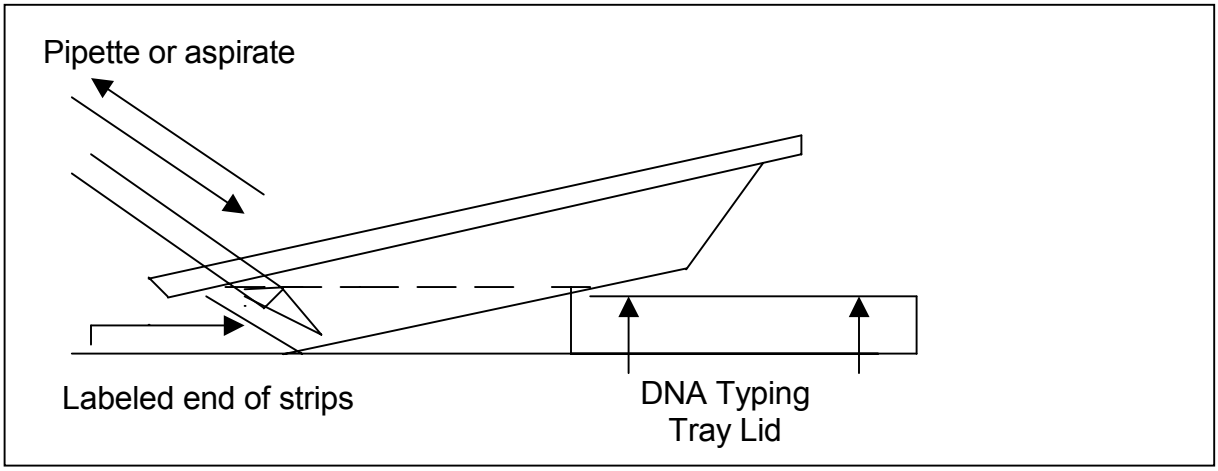

Figure 1 - Handling Solutions in DNA Typing Trays

H. Perform the following steps for each tube of amplified DNA.

NOTE: For each tube, perform** Steps H.1 **through** H.3 **within 20 seconds.

1. Remove the tube from the 95°C block of the GeneAmp® PCR Instrument System.
2. Carefully open the tube using the microcentrifuge tube de-capping device designated for use only with amplified DNA.
3. Withdraw 20 μl of amplified DNA from the aqueous (bottom) layer and immediately add it below the surface of the hybridization solution in the well of the corresponding probe strip (see ***Figure 1***).
4. Cap the tube after adding the denatured amplified DNA.
5. Repeat ***Steps H.1*** through ***H.4*** until each amplified DNA sample has been added to the corresponding well.

The remaining amplified DNA samples can be stored at 2° to 8°C for two weeks or at -20°C for 6 months. The continued acceptable performance of these samples beyond these periods may vary with the sample.

Store amplified DNA samples separate from all PCR amplification reagents, extracted DNA samples and casework samples.

I. Put the clear plastic lid on the tray and mix by carefully rocking the tray. Insure that each strip is completely wet. Once hybridization has begun, strips should remain wet through the conclusion of the Color Development and Photography steps.

J. Transfer the tray to the 55°C water bath. Place a 1 kg weight (e.g., a lead ring) on the covered tray to prevent the tray from sliding or floating. Check the tray position and insure that water does not splash into the wells of the tray. Adjust the water level if necessary. If water must be added to the bath, use water heated to 55°C (± 1ºC).

K. Replace the water bath cover to maintain the bath temperature at 55°C (± 1°C). Hybridize the amplified samples to the DNA probe strips by incubating at 55°C for 15 minutes (± 2 minutes).

L. Approximately 5 minutes **before the end** of the hybridization step, prepare the Enzyme Conjugate Solution in a glass flask using the following equations to determine the volume of each component required.

Number Hybridization Strips	x	*3.3 ml of Hybridization Solution*	=	*Volume of Solution*
Number of Strips	*x*	*27 µl of Enzyme Conjugate: HRP-SA*	=	*Volume of Enzyme Conjugate:HRP-SA*

Mix the solution thoroughly and insure that the solids remain in solution. Leave at room temperature (15° to 30ºC) until ready to use.

M. After hybridization remove the tray. Replace the water bath cover to maintain the temperature at 55°C (± 1ºC). Keep the water bath rotating between incubation steps.

N. Aspirate the contents of each well from the labeled end of the strip while tilting the tray slightly. Remove condensation from the tray lid with a clean lab wipe. Use of paper towels to wipe the tray lid is not advised because some paper towels contain bleach.

NOTE: PM + DQA1 Wash Solution solids must be completely dissolved and well mixed before use.

O. Dispense 5 ml pre-warmed PM + DQA1 Wash Solution into each well (a dispensing re-pipette is useful for this purpose). Rinse by rocking the tray for several seconds, then aspirate the solution from each well. Remove any solution from the tray lid with a clean lab wipe.

P. Dispense 3 ml of the Enzyme Conjugate Solution prepared in ***step L*** into each well and cover with the clear plastic lid. Transfer the tray to the 55°C water bath. Place a 1 kg weight on the covered tray to prevent the tray from sliding or floating. Adjust the rotating water bath to 50 to 90 rpm. Check the tray position and insure that water does not splash into the wells of the tray.

Q. Replace the water bath cover to maintain the temperature at 55°C (± 1°C). Incubate the Enzyme Conjugate Solution with the DNA probe strips at 55°C (± 1°C) for 5 minutes.

R. After incubation, remove the tray from the water bath, tip tray at a slight angle and aspirate the contents of each well from the labeled end of the strips. Remove condensation from the tray lid with a clean lab wipe.

NOTE: Replace the water bath cover to maintain the temperature at 55°C (± 1°C). Keep the water bath rotating between incubation steps.

S. Dispense 5 ml pre-warmed PM + DQA1 Wash Solution into each well. Rinse by rocking the tray for several seconds, then aspirate the solution from each well.

It is important to remove any solution from the tray lid with a clean lab wipe at this point to prevent excess blue background coloration from forming during the color development procedure.

T. To perform the stringent wash step, dispense 5 ml pre-warmed PM + DQA1 Wash Solution into each well. Cover the tray with the clear plastic lid and place it in the 55°C water bath. Place a 1 kg weight on the covered tray to prevent the tray from sliding or floating. Adjust the rotation to 50 to 90 rpm and check the tray position to insure that water does not splash into the wells of the tray.

U. Replace the water bath cover to maintain the temperature at 55°C (1°C).

Incubate the DNA probe strips at 55°C (± 1°C) for 12 minutes (± 1 minute).

The temperature and timing of the stringent wash step are critical.

V. After incubation, remove the tray from the water bath. Take off the lid and aspirate the contents of each well from the labeled end of the strips. Remove any liquid from the tray lid with a clean lab wipe.

W. Dispense 5 ml of PM + DQA1 Wash Solution into each well. Cover and rock the tray gently for several seconds.

X. Remove the lid and slowly pour off or aspirate the contents from each well into a designated waste container. Remove any solution from the tray lid with a clean lab wipe.

COLOR DEVELOPMENT

A. Dispense 5 ml Citrate Buffer into each well. A dispensing re-pipette is useful for this purpose. Cover the tray with the clear plastic lid and place it on an orbital shaker set at approximately 50 rpm at room temperature (15° to 30°C) for 5 minutes.

B. During this wash step, prepare the Color Development Solution.

Do not prepare the Color Development Solution more than 10 minutes before use.

Add the following reagents in the order listed to a glass flask and ***mix thoroughly by swirling*.**

Protect from light. Do NOT vortex.

Use the following equations to determine the volumes of each component required.

Number of Strips	x	*5 ml Citrate Buffer*	=	*Volume of Citrate Buffer*
Number of Strips	x	*5 µl 3% Hydrogen Peroxide*	=	*Volume of Hydrogen Peroxide*
Number of Strips	x	*0.25 ml Chromogen:TMB Solution*	=	*Volume of Chromogen:TMB*

C. Remove the tray from the orbital shaker. Remove the lid and slowly pour off or aspirate the contents from each well into a designated waste container. Add 5 ml freshly prepared Color Development Solution (***Step B***) to each well.

D. Develop the strips at room temperature (15° to 30°C) by rotating on an orbital shaker set at approximately 50 rpm for approximately 20 to 30 minutes.

NOTE: Place the clear plastic lid on the tray and cover the lid with aluminum foil during** Steps D, F, **and** G **to protect the DNA probe strips from strong light.

E. Remove the tray from the shaker. Remove the lid and slowly pour off or aspirate the contents from each well into a designated waste container.

F. Stop the color development by washing the strips in deionized or glass-distilled water (DI H_2O). Dispense approximately 5 ml DI H_2O into each well. Place the tray on an orbital shaker set at approximately 50 rpm for 5 to 10 minutes. Repeat ***Section E***.

G. Repeat ***Section F*** at least two times for a minimum of three DI H_2O washes. Additional 5 to 10 minute washes will reduce the potential for development of background color.

H. Record the pattern of blue dots from each wet AmpliType® PM DNA Probe Strip (see ***Interpretation of Results***).

NOTE: Keep strips wet throughout the photography steps.

PHOTOGRAPHY AND STORAGE

A. Photographs shall be taken for a permanent record of the results. ***Photographs must be taken while DNA probe strips are still wet***.

B. Place ***wet*** strips on a flat non-absorbent surface (a black background is recommended to enhance contrast). Keep the strips wet throughout the photography steps. Minimize exposure to strong light.

C. Use a Polaroid camera with Polapan 400 or Type 52 (black and white) film or Type 59 or 559 (color) film, or other suitable recording device.

> ***NOTE: For black and white photography, an orange filter (Wratten 22 or 23A) will enhance contrast.***

D. Follow film exposure and development instructions.

E. After photography, the DNA probe strips may be air-dried on any hard non-absorbent surface. Protect from light and oxidizing agents (e.g., acid treated paper, bleach, and nitric acid). The dot intensities may fade upon drying.

DISPOSAL OR REUSE OF TYPING TRAYS

A. The AmpliType® DNA Typing Trays are disposable or may be reused. Used trays and lids should be washed according to the following procedure.

> ***NOTE: Do NOT use detergent.***

1. To each well of the used AmpliType® DNA Typing Tray, add approximately 5 to 10 ml of 95% ethanol or 70% isopropanol.
2. Cover the tray with the lid and carefully agitate for 15 to 30 seconds to dissolve any residual Chromogen:TMB.
3. Remove the lid and pour off the ethanol or isopropanol from each well. Visually inspect each well for a faint blue color that will indicate the presence of Chromogen:TMB. If necessary repeat ***Steps A1*** and ***A2*** to remove any residual Chromogen:TMB.
4. Rinse each well in the tray and the tray lid with either glass-distilled or deionized ultrafiltered water. Repeat.
5. Dry trays before reuse.

INTERPRETATION OF RESULTS

Results are interpreted by observing the pattern and relative intensities of blue dots on the wet AmpliType® PM and AmpliType® HLA DQA1 DNA Probe Strips to determine which alleles are present in the DNA sample.

A. Reading and interpreting the AmpliType® PM + DQA1 DNA Probe Strips

The AmpliType® PM DNA Probe Strips have been spotted with a total of fourteen sequence-specific oligonucleotide probes to distinguish the alleles of five genetic loci (a mixture of two probes is spotted at the GYPA "A" allele position). Under the AmpliType® hybridization conditions, the typing probes will bind specifically to the PCR product containing the alleles designated on the AmpliType® PM DNA probe strip.

To read the developed AmpliType® PM DNA Probe Strip, the “S” dot is examined first and then each locus is examined separately. The standard probe “S” on the AmpliType® PM DNA Probe Strip is identical in sequence to the control probe “C” on the AmpliType® HLA DQA1 DNA Probe Strip and detects all of the HLA DQA1 alleles. The “S” dot is designed to be the lightest typing dot on the PM DNA Probe Strip and acts as a minimum dot intensity control for the remaining probes. A DNA probe strip with no visible “S” dot shall not be typed for any locus.

When an “S” dot is visible on the AmpliType® PM DNA Probe Strip, the intensities of the dots at the remaining 12 positions are compared to the intensity of the “S” dot. Those dots that appear either darker than or equivalent to the “S” dot are considered positive. Each positive dot indicates the presence of the corresponding allele. Dots that are lighter than the “S” dot should be interpreted with care.[14, 15]

The dots on the AmpliType® PM DNA Probe Strip correspond to the following alleles.

The “A” dot for each locus is positive in the presence of the A allele.

NOTE: The “A” dot for the GYPA locus is positive in the presence of both the A allele and the A’ allele. Both the GYPA AB and GYPA A and B variant alleles (observed in <8% of the African American population) may produce a slightly imbalanced heterozygous signal.

The “B” dot for each locus is positive in the presence of the B allele.

The “C” dot for the HBGG and GC loci is positive in the presence of the C allele.

For LDLR, GYPA, and D7S8, three genotypes are possible (AA, BB, and AB). For HBGG and GC, six genotypes are possible (AA, BB, CC, AB, AC, and BC).

An example of a developed AmpliType® PM DNA Probe Strip using PCR product amplified from 2 ng of Control DNA 1 is shown in ***Figure 2***. A sample from a single individual will produce balanced dot intensities within each locus for which the individual is heterozygous.

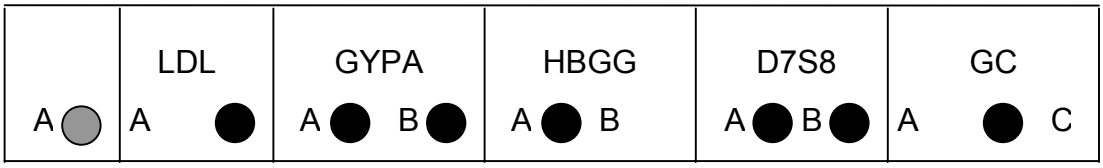

Figure 2 —AmpliType® PM types for Control DNA 1

The AmpliType® PM types for Control DNA 1 are: LDLR **BB**, GYPA **AB**, HBGG **AA**, D7S8 **AB**, GC **BB**. The dot intensities of the GYPA and D7S8 loci are balanced (i.e., the intensities of the "A" and "B" dots within each locus are similar).

B. Reading and Interpreting the AmpliType® HLA DQA1 DNA Probe Strips.

The AmpliType® HLA DQA1 DNA Probe Strips have been spotted with a total of eleven sequence-specific oligonucleotide probes to detect eight alleles of the HLA DQA1 locus. Under the AmpliType® hybridization conditions, the typing probes will bind specifically to PCR product containing the alleles designated on the AmpliType® HLA DQA1 DNA Probe Strip.

To read the developed AmpliType® HLA DQA1 DNA Probe Strip, the "C" dot is examined first and then the remaining dots are examined. The control probe "C" on the AmpliType® HLA DQA1 DNA Probe Strip detects all of the HLA DQA1 alleles. The "C" dot is designed to be the lightest typing dot on the strip and it indicates that adequate amplification and typing of the HLA DQA1 alleles in the sample have occurred. If the "C" dot is absent, an accurate determination of the type cannot be made. Additional information on the "C" dot can be found in the AmpliType® User Guide.[18]

The accurate interpretation of the HLA DQA1 results depends on the presence and intensity of the "C" dot. The intensities of the dots at the remaining ten positions are compared to the intensity of the "C" dot. Those dots that appear either darker than or equivalent to the "C" dot are considered positive. Each positive dot indicates the presence of the corresponding HLA DQA1 allele. Dots with signals less than the "C" dot should be interpreted with care.[9, 11, 28]

The dots on the AmpliType® HLA DQA1 DNA Probe Strip correspond to the following alleles.

The "1" dot is positive in the presence of the HLA DQA1 1.1, 1.2, and 1.3 alleles.

The "2" dot is positive only in the presence of the HLA DQA1 2 allele.

The "3" dot is positive only in the presence of the HLA DQA1 3 allele.

The "4" dot is positive in the presence of the HLA DQA1 4.1, 4.2, and 4.3 alleles.

Four HLA DQA1 sub-typing probes differentiate the HLA DQA1 1.1, 1.2, and 1.3 alleles.

The "1.1" dot is positive only in the presence of the HLA DQA1 1.1 allele.

NOTE: A faint "1.1" dot will appear with some HLA DQA2 pseudogene alleles.[30]

The "1.3" dot is positive only in the presence of HLA DQA1 1.3 allele.

NOTE: There is no probe that detects only the HLA DQA1 1.2 allele.

The "1.2, 1.3, 4" dot is positive in the presence of HLA DQA1 1.2, 1.3, 4.1, 4.2, and 4.3 alleles.

NOTE: The "1.2, 1.3, 4" dot can be lighter than the "C" dot when the genotype has an HLA DQA1 4.2 or 4.3 allele paired with an HLA DQA1 1.1, 2, 3, 4.2 or 4.3 allele because the HLA DQA1 4.2 and 4.3 alleles each have a single partially destabilizing mismatch to the "1.2, 1.3, 4" probe.[18] ***The partially destabilizing mismatch allows these two alleles to bind to this probe weakly relative to the HLA DQA1 1.2, 1.3, and 4.1 alleles.***

The "All but 1.3" dot is positive in the presence of all HLA DQA1 alleles EXCEPT 1.3. This probe is necessary to differentiate the 1.2, 1.3 genotype from the 1.3, 1.3 genotype.

NOTE: The "All but 1.3" dot can be equal to or lighter than the "C" dot when the genotype has an HLA DQA1 1.3 allele paired with an HLA DQA1 4.1, 4.2 or 4.3 allele because the HLA DQA1 4.1, 4.2, and 4.3 alleles have a single partially destabilizing mismatch to the "All but 1.3" probe.[18] ***The partially destabilizing mismatch allows these three alleles to bind to this probe weakly relative to the HLA DQA1 1.1, 1.2, 2, and 3 alleles.***

Two additional HLA DQA1 sub-typing probes differentiate the HLA DQA1 4.1 allele from the HLA DQA1 4.2 and 4.3 alleles.

The "4.1" dot is positive only in the presence of the HLA DQA1 4.1 allele.

The "4.2, 4.3" dot is positive in the presence of HLA DQA1 4.2 and 4.3 alleles.

An example of a developed AmpliType® HLA DQA1 DNA Probe Strip using PCR product amplified from 2 ng of Control DNA 1 is shown in ***Figure 3***. The AmpliType® HLA DQA1 type for Control DNA 1 is 1.1, 4.1.

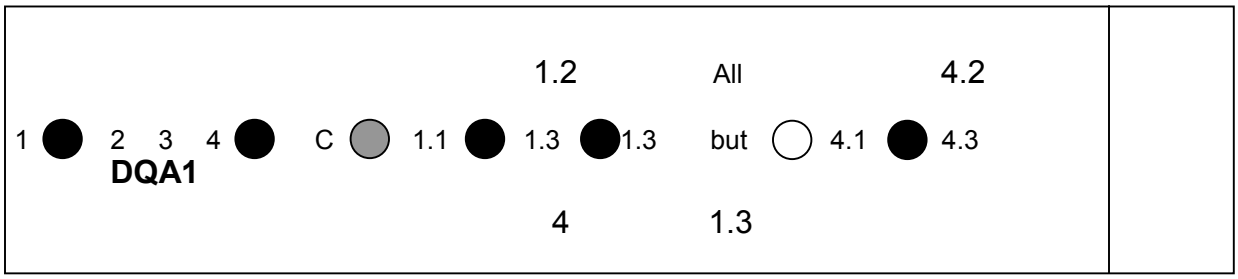

Figure 3 — The AmpliType® HLA DQA1 type for Control DNA 1

PERFORMANCE CHARACTERISTICS

The AmpliType® PM and AmpliType® PM + DQA1 PCR Amplification and Typing Kits are developed and manufactured by Roche Molecular Systems, Inc. (RMS). Each lot of the AmpliType® PM and AmpliType® PM + DQA1 PCR Amplification and Typing Kits is carefully tested by RMS to insure that the kits perform according to specifications and are free from interfering contaminants.

The user of the AmpliType® PCR Amplification and Typing Kits will be able to amplify and type a minimum of 2 ng of Control DNA 1 when employing the protocols and reagents provided in the kits.

In the laboratories of RMS, the kit components have been used successfully to type samples containing less than one ng of human DNA.

TROUBLESHOOTING

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
1. No signal or faint signal from both the Control DNA 1 and the DNA test samples at all loci.	No PCR amplification or insufficient PCR amplification of all markers.	Repeat test from the amplification step.
	Improper hybridization or assay condition.	Repeat test from hybridization step.
	No DNA added or insufficient DNA added to PCR Reaction Mix.	Add ≥ 2 ng DNA; repeat test from amplification step.
	AmpliType® PM Primer Set not added to PCR Reaction Mix.	Add AmpliType® PM Primer Set; repeat test from amplification step.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	GeneAmp® PCR Instrument System failure or wrong program.	*See* ***GeneAmp® PCR Instrument System Manual*** and check instrument calibration.
	Tubes not seated tightly in the DNA Thermal Cycler 480 block during amplification.	Push tubes firmly into contact with block after first cycle; repeat test.
	Hybridization or Stringent Wash temperature too high.	Check that the rotating water bath temperature is 55°C (± 1°C); repeat test.
	Hybridization or PM + DQA1 Wash Solution salt concentration too low.	Prepare new solutions; repeat test.
	Stringent Wash time too long.	Repeat test, washing for 12 minutes (± 1 minute) only.
	Amplified DNA was not added to DNA probe strips.	Repeat test, adding amplified DNA to DNA probe strips.
	Amplified DNA was not denatured.	Check GeneAmp® PCR Instrument System block temperature (95ºC) and leave sample in block >3 minutes; complete DNA addition to tray within 20 seconds for each sample; repeat test.
	Enzyme Conjugate:HRP-SA was not added to the Enzyme Conjugate Solution.	Prepare new diluted Enzyme Conjugate:HRP-SA solution; repeat test.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	Hydrogen peroxide was not added or too much was added to the Color Development Solution.	Make new Color Development Solution using 3% hydrogen peroxide; repeat test.
	Hydrogen peroxide inactive.	Make new Color Development Solution using new bottle or dilution of hydrogen peroxide; repeat test.
.	Chromogen:TMB was not added to the Color Development Solution.	Make new Color Development Solution adding Chromogen:TMB; repeat test.
	The original AmpliType® HLA DQα instead of AmpliType® PM and PM + DQA1 typing protocol was followed.	Repeat test following protocol in ***Section DNA Hybridization***.
2. Positive signal from Control DNA 1, but no signal from DNA test sample.	Quantity of DNA test sample is below the assay sensitivity.	Repeat amplification increasing DNA quantity to ≥2 ng.
3. High DNA probe strip background color.	Excess amounts of Enzyme Conjugate:HRP-SA added to Enzyme Conjugate Solution.	Dilute new Enzyme Conjugate Solution with correct amount of Enzyme Conjugate:HRP-SA. Cover tray with foil during Color Development steps. Repeat test using deionized or glass distilled water for water rinses.
4. High DNA probe strip background color upon storage.	Exposure to strong light and oxidizing agents.	Store strips in the dark away from oxidizing agents.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	Insufficient water washes after Color Development.	Increase number of water washes in future assays.
5. Presence of unexpected or additional alleles in the amplified Control DNA 1 sample.	Cross-hybridization caused by Hybridization or Stringent Wash temperature being too low.	Check that rotating water bath temperature is 55°C (± 1°C); repeat test.
	Cross-hybridization caused by Hybridization or PM + DQA1 Wash Solution salt concentration being too high.	Prepare new solutions; repeat test.
	Cross-hybridization caused by Stringent Wash time being too short.	Repeat test; washing for 12 minutes (± 1minute).
	Contamination by amplified product or samples.	*See* ***AmpliType® User Guide and References*** **18** ***and*** **28**.
6. Signals weaker than "S" or "C" dot on the same strip.	Hybridization or Stringent Wash temperature too high or too low.	Check that rotating water bath temperature is 55°C (± 1°C); repeat test.
	Hybridization or PM + DQA1 Wash Solution salt concentration too high or low.	Prepare new solutions; repeat test.
	Stringent wash time too long or short.	Repeat test, washing for 12 minutes (± 1 minute).
	Mixed sample or contamination.	*See* ***AmpliType® User Guide and References*** **14**, **15**, **17**, **18**, ***and*** **28**.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	Amplification of HLA DQA2 pseudogene (faint "1.1" dot).	*See* ***References*** **18** ***and*** **30**.
	EDTA not added to the reaction prior to the heat denaturation step of the DNA hybridization protocol.	Add EDTA to amplified sample (***Section PREPARATION OF PCR PRODUCT FOR DETECTION***); repeat test.
7. More than two alleles present on the AmpliType® HLA DQA1 DNA Probe Strip or at the HBGG and/or GC marker on the AmpliType® PM DNA Probe Strip.	Cross-hybridization caused by Hybridization and/or Wash temperature too low.	Check that the rotating water bath temperature is at 55°C (± 1°C); repeat test.
	Cross-hybridization caused by Hybridization and/or Stringent PM + DQA1 Wash Solution salt concentration too high.	Prepare new solutions; repeat test.
	Stringent Wash time too short.	Repeat test washing for 12 minutes (± 1 minute).
	Mixed sample or contamination.	*See* ***References*** **14, 15, 17, 18,** ***and*** **28.**
	Amplification of HLA DQA2 pseudogene (faint 1.1 dot).	*See* ***References*** **18** ***and*** **30.**
8. Some, but not all, loci visible on strips.	Degraded input DNA.	Evaluate the quality of the DNA sample. If the DNA is degraded, re-amplify with an increased amount of DNA.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	The test sample contains PCR-inhibitor.	*See* ***AmpliType® User Guide, Version 2, Section C***, for notes on DNA sample preparation.
	Input DNA and/or PCR product was not denatured sufficiently during amplification.	Check calibration of the GeneAmp® PCR Instrument System Temperature Verification System.
	Amplified DNA was not denatured.	Check GeneAmp® PCR Instrument System block temperature (95°C) and leave sample in block >3 minutes; complete DNA addition to tray within 20 seconds; repeat test.
9. Imbalanced dot intensities within a locus (the kit is designed to produce balanced dot intensities when heterozygous samples are typed, except as described in ***Observation 12.***).	Hybridization or Stringent Wash temperature too high or too low.	Check that rotating water bath temperature is 55°C (± 1°C); repeat test.
	Hybridization or PM + DQA1 Wash Solution salt concentration too high or too low.	Prepare new solutions; repeat test.
	Stringent Wash time too long or too short.	Repeat test; washing for 12 minutes (± 1 minute).
	Mixed sample or contamination.	*See* ***References* 14, 15, 17, 18, *and* 28.**

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED ACTION
	EDTA not added to the reaction prior to the heat denaturation step of the DNA hybridization protocol.	Add EDTA to amplified sample; repeat test.
10. Weak or absent "4.1" dot on the AmpliType® HLA DQA1 DNA Probe Strip in the amplified Control DNA 1 sample.	EDTA not added to the reaction prior to the heat denaturation step of the DNA hybridization protocol.	Add EDTA to amplified sample; repeat test.
11. "1.2, 1.3, 4" dot weaker than "C" dot on AmpliType® HLA DQA1 DNA Probe Strip.	Genotype of sample has a HLA DQA1 4.2 or 4.3 allele paired with a HLA DQA1 1.1, 2, 3, 4.2, or 4.3 allele.	*See* ***Reference* 18.**
12. "1.1" dot weaker than "C" dot but no signal for "1' dot on AmpliType® HLA DQA1 DNA Probe Strip.	Amplification of HLA DQA2 pseudogene (faint 1.1 dot).	*See* ***References* 18 *and* 30.**
13. "All but 1.3" signal weaker than "C" dot on AmpliType® HLA DQA1 DNA Probe Strip.	Genotype of sample has a HLA DQA1 1.3 allele paired with a HLA DQA1 4.1, 4.2, or 4.3 allele.	*See* ***Reference* 18.**

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ALLELE FREQUENCIES – DQA1, PM

Table 2: HLA-DQA1 observed allele frequencies in several United States general population groups

	African Americans (N = 491)	Caucasians (N = 363)	Southwestern Hispanics (N = 333)
Allele 1.1	0.127	0.153	0.122
Allele 1.2	0.292	0.198	0.131
Allele 1.3	0.046	0.065	0.042
Allele 2	0.102	0.152	0.111
Allele 3	0.109	0.174	0.230
Allele 4.1	0.202	0.234	0.255
Allele 4.2/4.3	0.122	0.025	0.110

N refers to the number of individuals in the database.

Table 3: Observed allele frequency distributions for PM loci in several United States general population groups

Allele	African American (N=636)	Caucasian (N=511)	Southwestern Hispanic (N=304)
LDLR A	0.192	0.432	0.535
LDLR B	0.808	0.568	0.465
GYPA A	0.502	0.558	0.641
GYPA B	0.498	0.442	0.359
HBGG A	0.439	0.465	0.337
HBGG B	0.236	0.532	0.635
HBGG C	0.324	0.003	0.028
D7S8 A	0.645	0.602	0.617
D7S8 B	0.355	0.398	0.383
Gc A	0.100	0.280	0.227
Gc B	0.725	0.155	0.250
Gc C	0.175	0.566	0.523

N refers to the number of individuals in the database.

SHORT TANDEM REPEAT ANALYSIS

PRINCIPLES OF STR ANALYSIS CASEWORK

AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® Cofiler™

The AmpFℓSTR® Profiler Plus™ (Profiler Plus or PP) and AmpFℓSTR® Cofiler™ (Cofiler or CO) DNA typing systems (Applied Biosystems) utilize the polymerase chain reaction (PCR) to amplify regions of DNA known as short tandems repeats (STRs) in order to characterize DNA extracted from forensic specimens. Profiler Plus and Cofiler are multiplex systems which allow for the simultaneous amplification of numerous STR loci as well as a portion of the amelogenin gene located within the X and Y chromosomes. Analysis of amelogenin allows for gender determination. The Profiler Plus and Cofiler kits contain all reagents needed for amplification, which includes: primer sets which are specific for the various loci, PCR reaction buffer, and AmpliTaq Gold® DNA polymerase as well as the required allelic ladders. The primer sets contained within each kit consist of both unlabeled primers and those that are labeled with one of three distinctive fluorescent dyes. The use of multicolor dyes permits the analysis of loci with overlapping size ranges. The amplified fragments are separated according to size by capillary electrophoresis using the ABI Prism™ 310 Genetic Analyzer. The amplified fragments are detected by laser excitation. A charged coupled device (CCD) camera displays the signals as peaks and then captures the subsequent emission spectra. The resulting data will be graphically displayed as colored peaks noted by height in relative fluorescent units (RFUs) and time (scan number). This display is called an electropherogram.

The reference allelic ladders for each of the STR loci and reference fragments for amelogenin are also subjected to capillary electrophoresis. These allelic ladders contain the more common alleles in the general population for each locus. Using the ladders, the alleles present in known and questioned DNA specimens may be determined.

The following table lists the Profiler Plus and Cofiler loci, the size ranges of alleles within a particular locus, the alleles present in the ladder, the color of the labeled primer and the DNA profile of the Positive control (9947A). Note that some of the same loci are amplified in both kits.

Additional information, procedures, and protocols may be relied on from the **Federal Bureau of Investigation** *Short Tandem Repeat Analysis Protocol (*July 28, 2000), the **ABI PRISM™ 310 Genetic Analyzer** *User's Manual* (1998), **ABI PRISM™ 310 Genetic Analyzer** *GeneScan Reference Guide* (1997), **GeneAmp® PCR SYSTEM 9700** *User's Manual Set* (1997), **Perkin-Elmer DNA Thermal Cycler 480** *Quick Reference Guide* (1994), and the **AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® Cofiler™** *User's Manual* (1998).

Table 4

SYSTEM	LOCUS	SIZE RANGE (bp)*	ALLELES PRESENT IN LADDER[1]	DYE COLOR	POSITIVE CONTROL 9947A
Both	D3S1358	111-140	12 – 19	FAM-5 Blue	14, 15
Profiler Plus	vWA	154-195	11 - 21	FAM-5 Blue	17, 18
Profiler Plus	FGA	216-265	18-26, 26.2, 27-30	FAM-5 Blue	23, 24
Both	Amelogenin	103, 109	X, Y	JOE Green	X, X
Profiler Plus	D8S1179	124-171	8 - 19	JOE Green	13, 13
Profiler Plus	D21S11	187-240	24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 38	JOE Green	39, 30
Profiler Plus	D18S51	270-342	9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-26	JOE Green	15, 19
Profiler Plus	D5S818	131-170	7 - 16	NED Yellow	11, 11
Profiler Plus	D13S317	205-233	8 – 15	NED Yellow	11, 11
Both	D7S280	256-293	6 – 15	NED Yellow	10, 11

[1] The allelic ladders provided in the kit are used to determine the genotypes of the samples.

Table 4

SYSTEM	LOCUS	SIZE RANGE (bp)*	ALLELES PRESENT IN LADDER[1]	DYE COLOR	POSITIVE CONTROL 9947A
Cofiler	TH01	167-187	5-9, 9.3, 10	JOE Green	8, 9.3
Cofiler	TP0X	215-243	6 – 13	JOE Green	8, 8
Cofiler	CSF1P0	279-318	6 – 15	JOE Green	10, 12
Cofiler	D16S539	229-270	5, 8-15	FAM-5 Blue	11, 12

*Sizes are actual base pair size of alleles in the Profiler Plus and Cofiler ladders (including the nucleotide addition). The sizes obtained in **Genotyper** may vary from the actual fragment sizes due to electrophoretic effects.

GUIDELINES FOR CONTROL SAMPLES

It is imperative that proper control samples be included when evidence samples are extracted, quantified, amplified, and typed through capillary electrophoresis. The typing results obtained from these control samples are essential for the interpretation of STR and amelogenin typing results from evidentiary samples. The controls also serve to assess the quantity and quality of the extracted DNA as well as the effectiveness, accuracy and precision of the analytical procedures.

EXTRACTION CONTROLS

Reagent Blank

With each set of extractions of evidentiary samples a reagent blank must be included. The reagent blank consists of all reagents used in the test process minus any sample and is processed through the entire extraction, quantification, amplification, and electrophoretic typing procedures. If more than one type of extraction procedure is employed (with different extraction reagents) then a reagent blank must be set up for each type of extraction or group of extraction reagents used. Twenty microliters of the reagent blank should be amplified. The reagent blank must be run in at least one of the PCR systems to check for contamination of the reagents with genomic DNA.

Extraction/Allelic Control

The purpose of this control is to insure that the extraction and typing procedure worked properly. The control may be either a bloodstain or other biological material from a previously characterized source that suitably resembles samples extracted. It is extracted along with the casework samples using the same reagents and is NIST

traceable. The alleles for each locus are documented in the ***CBI Forensic Laboratory NIST Logbook***.

AMPLIFICATION CONTROLS

Negative Amplification Blank

A Negative Amplification Blank must be used with each set of amplifications. The negative amplification blank contains all components required for the amplification of DNA except that no DNA is added. Twenty microliters of TE Buffer is placed in the negative amplification blank in lieu of a DNA solution. This control is processed through the amplification and capillary electrophoretic typing procedures.

Positive Amplification Controls

Cell line DNA 9947A is a positive control for STRs and amelogenin to evaluate the performance of amplification and capillary electrophoresis. When the control specimen 9947A is amplified, the STR loci must solely exhibit the correct genotype. Additionally, specimen 9947A is the female control for amelogenin and must exhibit a single band at the position corresponding with the size ladder band representative of the 103 bp peak from the X chromosome.

EQUIPMENT

Thermal Cycler 480 and 9700

The DNA thermal cycler (480 or 9700) can be used to amplify both Chelex™ extracted and organic extracted casework samples; the thermal cycler is evaluated biannually.

ABI PRISM™ 310 Genetic Analyzer

The ABI Prism™ 310 Genetic Analyzer is an automated capillary electrophoresis system. This system can separate, detect, and analyze fluorescent labeled DNA fragments. The system includes an ABI prism™ Genetic Analyzer, collection software and computer workstation with MAC operation system and GeneScan analysis software.

Matrix

A new matrix will be created for each instrument whenever analytic data indicates the necessity (on a semiannual basis, at minimum). Refer to *How to Create the GeneScan Matrix File* in the "GeneScan Analysis Software Experiments" of the *ABI PRISM™ 310 Genetic Analyzer User's Manual* for instructions on how to build a matrix. Once prepared, a matrix will be considered suitable for casework if a flat baseline is obtained when it is applied to the matrix standards.

NOTE: ***A new matrix must be built following cleaning, maintenance, or replacement of a component of the optics.***

Capillary

A new capillary will be installed whenever analytic data indicates the necessity (approximately 300 injections).

Buffer and Waste Vials

The cathode buffer and waste vials will be replaced, at a minimum, every 72 hours.

Polymer

The POP-4 will be replaced, at a minimum, every 72 hours.

Power Macintosh® Computers

Hard drives will be defragmented periodically using Norton Utilities to insure efficient computer operation.

QUALITY CONTROL OF CRITICAL SUPPLIES AND REAGENTS

Prior to being used for case analysis, certain supplies and reagents should be checked against materials known to give an acceptable result. The testing procedures for these items are given below.

AMPLIFICATION REAGENTS

Profiler Plus and Cofiler Kits

Each lot of kits will be evaluated using the extraction control that has been designated to be NIST traceable. The criteria for kit quality sufficient for use in casework are:

A. a minimum of 0.75 ng sensitivity and

B. correct genotypes obtained for the NIST traceable CBI internal control or extraction control. The genotypes for the NIST traceable CBI DNA controls will be kept in the logbook with the results of the kit validations.

The positive amplification control (9947A) will be suitable for use in case work if complete and correct typing of this sample is obtained.

The allelic ladders are suitable for use in case work if:

C. each ladder allele is greater than 100 RFU and

D. correct typing results are obtained for 9947A when the ladders are employed in Genotyper.

QUANTIFICATION PROCEDURE

An estimation of the DNA content of the samples will be conducted prior to STR analysis. CBI uses the QuantiBlot® biotin-streptavidin-HRP method to evaluate the DNA concentration of each extracted sample. In general, 0.75 to 2.0 ng of DNA is optimal for STR amplification, although correct results may be obtained outside of this range.

AMPLIFICATION

All PCR setup steps must be performed in the DNA Laboratory using hoods, reagents, and pipettors dedicated to this area.

The reagents required for the amplification of the thirteen core STR loci are included in two kits, the AmpFℓSTR® Profiler Plus™ PCR Amplification Kit, and the AmpFℓSTR® Cofiler™ PCR Amplification Kit. For each amplification, combine DNA template, PCR reaction mix, Primer set (Profiler Plus or Cofiler) and DNA polymerase (AmpliTaq Gold®) in a reaction tube and subject this mixture to a series of controlled temperature changes in the Thermal Cycler.

Thermal Cycler Parameters

The following parameters are used to amplify the AmpFℓSTR® Profiler Plus and Cofiler loci on the PE Biosystems thermal cyclers.

Turn on the Thermal Cycler and allow the instrument to warm up for a minimum of 1 hour. Add one drop of oil (480's only) into each of the wells to be used. Select the file for forensic STR analysis. The parameters are listed below.

DNA Thermal Cycler 480 or 9700

Pre-heat (step cycle file, 1 cycle):	95ºC,	11 minutes
Cycle (step cycle file, 28 cycles):	94ºC,	1 minute
	59ºC,	1 minute
	72ºC,	1 minute
Final extension (step cycle file, 1 cycle)	60ºC,	90 minutes
Hold temperature (soak file)	10ºC,	indefinite

Tubes can be left in the Thermal Cycler at 10°C until removed. They can then be stored at 2-6°C for a week, or longer at -15 to -20°C until processed and analyzed on the 310.

SAMPLE PREPARATION

A. Determine the number of samples to be amplified. Place the required number of amplification reaction tubes into a rack and label them.

B. Fill out a STR dilution sheet.

C. Samples will be diluted as necessary, according to instrument sensitivity. Twenty microliters of this solution will be added to the Profiler Plus Master mix in the appropriate amplification tube, and another 20 μl will be added to the Cofiler Master Mix.

AMPLIFICATION MIX PREPARATION

The following formulation applies to both the Profiler Plus and Cofiler kits.

A. Vortex at medium speed the PCR reaction mix, primer set and AmpliTaq Gold® DNA polymerase reagents supplied in the kit for 5 seconds. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

B. In 1.5 ml microcentrifuge tube put:

   1. (Number of samples +1) x 21.0 μl of PCR reaction mix,
   2. (Number of samples +1) x 1.0 μl of AmpliTaq Gold® DNA polymerase, and
   3. (Number of samples +1) x 11.0 μl of primer set.

C. The above formulation provides a slight overfill to allow for volume lost in pipetting. The maximum volume of Master Mix held in a 1.5 ml tube can be dispensed into 42 PCR tubes. A 2.0 ml tube is recommended when preparing Master Mix for up to 55 samples. Be sure to include enough Master Mix for reagent blanks, a positive control, and a negative amplification blank.

D. Mix thoroughly by vortexing at medium speed for 5 seconds.

E. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.

F. Dispense 30 μl of Master Mix into each GeneAmp® Thin-Walled Reaction tube.

G. If using the 480 thermal cycler, add one drop mineral oil (supplied with kit) to each of the tubes. (This step not necessary if using the 9700 thermal cycler).

H. To each forensic-sample tube, add 20 μl of sample DNA/TE sample preparation (insert the pipette tip below the oil layer if using the 480 thermal cycler). After the addition of sample, cap the tube before proceeding on to the next tube. Do not mix or vortex the tube. The final PCR reaction volume is 50 μl.

I. Add 20 μl of the reagent blank to a tube for the reagent blank control.

J. Add 20 μl of the diluted positive control DNA provided in the kit in a concentration consistent with the amount of DNA used in the forensic samples to a tube for the positive control.

K. Add 20 μl TE Buffer to a tube for the negative amplification blank.

L. Place the PCR tubes in the thermal cycler and start the program. If using the 480, record the heat block position of each tube.

M. After the amplification process, remove the tubes from the thermal cycler and store the amplified products protected from light.

N. The amplified products can be stored at 2 to 6°C for no more than 2 weeks. For longer periods, store the tubes at -15 to -20ºC.

STR TYPING BY CAPILLARY GEL ELECTROPHORESIS

SETTING UP THE INSTRUMENT

A. Turn on the ABI PRISM™ 310 Genetic Analyzer.

B. Restart the Power Macintosh® computer. Launch the ABI 310 Collection software if it is not already open.

Note: It is not necessary to change the capillary for every run. The maximum # of injections per capillary is approximately 300.

C. Select Change Capillary from the Instrument menu to determine whether the capillary needs to be changed. Select Cancel to close.

D. Remove the POP-4™ from the refrigerator to warm to room temperature. If precipitate is present when the bottle is removed, warming to room temperature and gentle mixing (rotate slowly by hand carefully so as to not introduce air into the POP-4™) will remove the precipitate. If the precipitate will not dissolve, do not use the POP-4™.

E. Home the instrument as follows.

1. Under Windows menu select Manual Control.
2. Select Syringe Home from the Function pop-up menu and click Execute.
3. Select Autosampler Home X, Y-Axis from the Function pop-up menu and click Execute.
4. Select Autosampler Home Z-Axis from the Function pop-up menu and click Execute.

F. Installing a New Electrode (If this procedure is not necessary, proceed to ***Step G***).

1. Unscrew the electrode thumbscrew located to the right of the detector door and just below the heat plate.
2. Insert the long end of the new platinum electrode into the electrode hole of the thumbscrew.
3. Insert the hooked end of the electrode into the outer hole.
4. Replace the electrode back on the instrument.
5. Under the Manual Control window, select Autosampler Home Z-Axis from the Function pop-up menu, click Execute.
6. Use flush-cutting wire cutters (with the flat cutting surface facing the top of the instrument) to cut off a small piece of the electrode so that it is flush with the lower surface of the stripper plate.

G. Removing and cleaning the syringe.

1. If the syringe drive is not in the home position, select Syringe Home from the Function pop-up menu of the Manual Control window.
2. Move the drive toggle to the left position to allow for removal of the syringe from the pump block.
3. Unscrew the syringe to remove it from the pump block.
4. Clean and rinse the 1.0 ml syringe with warm tap water (if necessary), then with filter-purified water. Remove all excess water from the inside and outside of the syringe using compressed air.

H. Priming the syringe.

1. Draw up 1.0 ml of filter-purified water and then expel.
2. Draw up approximately 0.2 ml of room temperature POP-4™ into the syringe. Pull plunger so that the tip is drawn to the 0.8 to 1.0 ml mark. Remove air bubbles from the tip and slowly expel the POP-4™ through the syringe body and finally expel the POP-4™ onto a Kimwipe or waste container.

I. Filling the syringe.

1. Fill the 1.0 ml syringe to a maximum of 0.8 ml of POP-4™ polymer.

NOTE: The following formula can be used as a guideline for the approximate amount of polymer to sample.

2. (Sample # x 6 µl POP-4™) + 200 µl POP-4™.

NOTE: Polymer that has been on the instrument for more than 72 hours should not be used for analysis. Do not return the unused polymer to the original bottle.

3. Rinse the outside of the syringe with distilled water and dry with a Kimwipe to remove excess polymer.
4. Remove any air bubbles by inverting the syringe and pushing out a small amount of polymer.

NOTE: It is critical that all air bubbles be expelled from the syringe.

J. Cleaning the Pump Block.

1. Within the Manual Control window, select Syringe Home from the Function pop-up menu. Select Buffer Valve Open, press Execute.
2. Unscrew the glass syringe.
3. Remove any previously installed capillary.

NOTE: If the capillary has been used for less than 300 injections, it may be stored with both ends in 1x Buffer or ultrapure H_2O.

4. Grasp the block with both hands and pull straight out.
5. Rinse the block, valves, and ferrule thoroughly with warm tap water (if necessary), then with filter-purified water.
6. Remove any excess water from inside and outside of the block using compressed air.
7. Verify that the gold electrode socket on the back of the block is dry and then replace the pump block. Slide the U-shaped end of the activator arm into the collar at the top of the plunger valve. Make sure the activator arm lines up with the groove in the pin valve before pressing into place. It may be necessary to move the activator arm up or down by selecting Buffer Valve Open or Closed from the Function pop-up menu and selecting Execute.

K. Installing the Syringe on the Pump Block.

1. Move the syringe drive toggle on the subject pump drive to the left to allow for replacement of the syringe to the pump block.
2. Place the 1.0 ml syringe in through the right-hand port of the syringe guide plate and screw it into the pump block. The syringe should be finger-tight in the block.

NOTE: Tighten the syringe at the metal collar. Excess pressure on the glass syringe barrel will break the seal of the collar, permanently damaging the syringe.

3. Hand-tighten the valves on the pump block to the left of and below the syringe.

L. Installation of a New Capillary.

1. Remove the new capillary from the plastic sleeve and clean the detection window with ethanol and lens paper.
2. Open the door covering the heat plate, and then partially unscrew the plastic ferrule on the right side of the pump block.
3. Thread the capillary through the center of the ferrule.
4. Tighten the ferrule in the block. As the ferrule begins to seat, adjust the end of the capillary so that it is positioned directly below the opening of the glass syringe. With buffer valve closed, push polymer down into block. Allow to flow into capillary opening. Finger-tighten the ferrule to secure the capillary.
5. Open the laser detector door and position the capillary in the vertical groove of the detector. Position the center portion of the capillary detection window in the laser detector window.

6. Close the laser detector door.
7. Thread the other end of the capillary through the capillary hole in the electrode thumbscrew until it extends approximately 0.5 mm beyond the electrode.
8. Secure the capillary in place by taping it to the heat plate above the laser detector door and above the electrode.
9. Close the heat plate door.

NOTE: The capillary injection counter must be reset each time a new capillary is installed.

M. Fill the Buffer Reservoirs.

1. Dilute 1.5 ml of 10X Genetic Analyzer Buffer with EDTA up to 15 ml (makes a 1X solution) with filter-purified water.

NOTE: A larger volume can be made if desired. The 1X solution can be stored at 4ºC for up to 2 weeks.

2. Fill the anode buffer reservoir to the red line with 1X Genetic Analyzer Buffer with EDTA and replace the reservoir on the pump block.
3. Fill a 4 ml glass buffer vial to the full line with 1X Genetic Analyzer Buffer with EDTA. Insert the plastic vial lid (with attached septum) into the glass vial. Place this buffer vial in position 1 on the Autosampler. This is the cathode buffer.
4. Fill a second 4 ml glass buffer vial to the fill line with filter-purified water. Insert the plastic vial lid (with attached septum) into the glass vial. Place this buffer vial in position 2 of the Autosampler.
5. Fill a 1.5 ml microcentrifuge tube (cut off the lid) with filter-purified water and place into position 3 on the Autosampler. Do not use a screw-top tube.

NOTE: To avoid electrical arcing, it is imperative that all surfaces of the buffer vials and the microcentrifuge tube be clean and dry.

N. Calibrating the Autosampler.

NOTE: Autosampler calibration should be performed whenever the capillary or electrode is changed or whenever the position of the electrode is altered.

1. Choose Autosampler Calibration from the Instrument menu.
2. Select Start, remove Autosampler tray if necessary, and select Resume.
3. Follow the instructions on the screen to set the calibration point. Click the direction arrows to align the front calibration point of the Autosampler with

the capillary. To make fine adjustments, press the shift key while clicking the direction arrow. The end of the capillary must be centered on and nearly touch the calibration point.

4. When complete, select Done.

5. From the menu select Manual Control and select Autosampler to Position from the pop-up menu. Type a 1 in the value box and select Execute. Then select Autosampler Up and Execute until the capillary is inside the 1x buffer.

O. Prime the pump block.

1. Select Buffer Valve Close from the Function pop-up menu and click Execute.

2. Open the waste valve below the syringe by partly unscrewing it.

3. Push down on the syringe until the valve space is filled with polymer and then tighten the valve, making sure no air is in the pump block space above the waste valve.

4. Open the valve to the left of the syringe and push polymer through to that valve region.

5. Push down on the syringe until the valve space is filled with polymer and then tighten this valve, making sure no air is in the pump block space above the waste valve.

6. Select Buffer Valve Open from the Function pop-up menu and click Execute.

7. Press down on the syringe in order to push polymer through the remainder of the block channel. Observe as the bubbles travel through and out of the block and a drop of polymer falls through the anode buffer.

8. Select Buffer Valve Close from the Function pop-up menu and click Execute. There should be no bubbles in the block channels.

P. Seat the syringe drive.

1. Select Buffer Valve Open from the Function pop-up menu and click Execute.

2. Move the syringe toggle to the right so that it is positioned over the syringe plunger.

3. Select Syringe Down from the Function pop-up menu and enter a value from 200 to 5 and click Execute. Repeat this procedure until the toggle seats on the plunger. (Use smaller increments the closer the toggle comes to the plunger.)

Q. Set the temperature.

1. Close the instrument doors.
2. Select Temperature Set from the Function pop-up menu and enter 60 in the Value box. Click Execute. It takes approximately 30 minutes for the instrument to reach the run temperature of 60ºC.

PREPARING FOR A RUN

WARNING!! CHEMICAL HAZARD!! Formamide is an irritant and a teratogen. Avoid skin contact and inhalation. Use in a well-ventilated area. Wear lab coat and gloves when handling.

A. Remove deionized formamide from the freezer to thaw.

B. Prepare a sample sheet.

1. Choose New from the file menu.
2. Click on the appropriate GeneScan Sample Sheet icon.

   Fill in the appropriate information as defined by current software directions.
3. Fill in the sample name column first. When complete, highlight the Sample Name column by clicking once in the gray bar at the top and select Copy from the Edit menu.
4. Highlight the Sample Info column and select Paste from the Edit menu.
5. Insure there is a diamond next to the red box in the Std column for each sample.
6. Make sure there is an X in each column for the Pres column.

NOTE: ***The sample name must be in accordance with the guidelines in*** **CODIS Standard Operating Procedures.** ***Refer to "CODIS Identification Number" in Section III. Insure that the information contained under the Sample Name column is copied onto the Sample Info column. (See*** **section** *Electronic File Naming Convention for the 310 STR Analysis*.)

Note: Insure that the Std column highlights red.

Note: Under the Sample Info column insure that each sample has a unique sample number, also insure that each lane which contains an allelic ladder has the word "ladder" in the Sample Info column; this is necessary for automated allele calling with Genotyper (v. 2.0 or higher)

7. Select Save As from the File menu and label the sample sheet "SS'date' analyst's initials PP and/or CO" e.g. "SS01/22/00xyzPP/CO" or "SS012200xyzPPCO."

C. Prepare the samples for analysis.

1. Remove the GeneScan-500 [ROX] internal lane standard and allelic ladders from the refrigerator. Vortex and spin.
2. Add GeneScan-500 [ROX] to the thawed formamide at a concentration necessary to obtain an RFU of 750 to 3000 in the blank.
3. Vortex the tube and spin in a centrifuge.
4. Place the appropriate number of sample tubes in the tray and label appropriately.
5. Add to each tube 25 μl of the formamide-ROX mixture.
6. Following the Sample Sheet format add samples as follows.
   a. Cap a tube with septum (BLANK).
   b. Add to two tubes: 1.5 μl of the appropriate allelic ladder and cap with septum.
   c. Add 1.5 μl of each sample into the appropriate tube and cap with septum before going to the next tube.

   NOTE: Septa or Septa Strips melt at high temperatures. Do not close the lid of the 9700 Thermal Cycler when denaturing — the septa will adhere to the lid. Do not autoclave or re-use septa.
7. Make sure that samples are mixed thoroughly and without bubbles on the bottom of the tube. (Vortex each sample and spin in a centrifuge.)
8. Denature the samples at 95ºC for 3 minutes (no more than 5 minutes).
9. Snap cool on ice-water bath (4ºC) immediately for a minimum of 3 minutes.
10. Secure the septa.
11. Replace samples in the tray. Under the Manual Control window, select "Present Autosampler" followed by Execute. Open the door and place tray onto the Autosampler with position A1 at the upper right-hand corner. Return tray to position (Manual Control – Autosampler Return – Execute). Close door.

D. Prepare notes sheet. Include the following information as a minimum.

1. Instrument ID.
2. Capillary lot #.
3. Buffer lot #.
4. POP-4™ lot #.

5. Formamide lot#
6. Cases included in the run.
7. Any other additional comments associated with the run may be added to the note section.

E. Fill out an Injection List.

1. Select New from the ABI collection File menu. Click on the GeneScan Injection List icon.
2. In the Injection List window, which appears, select the appropriate Sample Sheet pop-up menu.
3. Insure that the correct Module was inserted into the Injection List. The correct parameters for Module GS STR POP4™ (1ml) F are:

   a. Inj. Secs: 5,

   b. Inj kV: 15.0,

   c. Run kV: 15.0, and

   d. Run ºC: 60.

NOTE: If it is necessary to re-inject a sample due to off-scale data, the injection time may be decreased.

   e. Set the run time to insure that the necessary base pair peaks are captured (e.g. 24-30 minutes to obtain the 75 base pair to 400 base pair peaks of the GS500).

NOTE: The time necessary to obtain the 400 base pair peak can be set as a default module for the instrument.

   f. For additional injections highlight the Inj.# and select *Command*-I from the keyboard. Highlight the sample row and from the pop-up menu select the desired sample.

   g. It is recommended that the first two injections should be from the BLANK tube. Run a “Seq. Fill Capillary” with one injection especially if it is a new run and/or new polymer was placed in the syringe. If desired, a third blank can be run at the start of the run.

   h. It is recommended that the positive control, negative control, and reagent blank be injected twice on each run.

   i. Bracket each run of samples with the appropriate ladder (Profiler Plus or Cofiler) to insure at least one comparable ladder is available for each sample. This may require that the ladder be injected several times throughout the run.

NOTE: It may be beneficial to prepare two ladder tubes for each run.

NOTE: The Matrix column should indicate either <none> or the name of the matrix made.

j. Fill in the Operator's name and click the Run button.

NOTE: If necessary, additional injections can be added to the analysis run during the run.

F. 310 analysis run complete.

1. Once the run is completed, save the Injection List and the Analysis Run.
2. Save the Analysis folder as follows: RunFolder'date'analyst's initialsPP (and/or CO), e.g. run folder 01/22/00xyzPP or run folder 012200xyzPP.
3. Once saved, transfer the folder along with the notes and other necessary data, to an analysis computer.
4. From the Manual Control, select the Present Tray from the pop-up menu.
5. Remove the tray and from the Manual Control, select the Return Tray from the pop-up menu, then click Execute.
6. If completed with all runs the instrument will be powered down by:
   a. discarding buffer vials,
   b. cleaning the pump block,
   c. placing the capillary into two tubes of buffer or filter-purified water and store until used again, and
   d. turning off the computer.
7. If running additional samples:

a. replace the buffer and water vials if necessary (>72 hours),
   b. replace the water waste tube, and
   c. add POP-4™ if necessary.

GENESCAN/GENOTYPER ANALYSIS

GeneScan analysis software is used to analyze the raw data collected by the CE310 Genetic Analyzer. Genotyper software is used to automatically convert allele sizes from GeneScan Analysis software into allele designations and to build tables containing Genotyper information as needed. Genotypes are assigned by comparing the sizes obtained for the unknown sample alleles with sizes obtained for the alleles in the allelic ladder.

Utilizing the GeneScan and Genotyper analysis software, determine the allele designation for each sample. The following criteria must be accomplished during the use of the GeneScan and Genotyper analysis software (refer to the appropriate software manuals as needed):

A. Apply correct matrix

B. Insure that red dye color is marked as the size standard

C. Define and apply a size standard file with the peaks for 75, 100, 139, 150, 160, 200, 300, 340, 350, and 400 (Profiler Plus) base pair peaks labeled

D. Define and apply an analysis parameter with the following criteria.

   1. Analysis range: incorporate 75 to 400 base pair range
   2. Data processing: check baseline, multi-component, and light smoothing
   3. Peak detection: B50, G50, Y50, and R150 RFUs. The RFU may be increased to reduce signal to noise ratios.
   4. Size call range: min 75, max 400
   5. Size calling: local southern method
   6. Split peak correction: none

E. Verify all peaks in the GS500 size standard have been correctly assigned for each sample and that the 250 peaks throughout the analysis sample set are within +/- one base pair.

F. Examine reagent blanks and negative amplification blanks for anomalous peaks

G. Examine each sample making note of any anomalies (pull-up, spikes, -A peaks, dye peaks, peak height variation, etc.) or other electrophoretic aberrations (noisy baseline, matrix quality, incomplete electrophoresis etc.).

H. Examine and check to make sure all ladder alleles are labeled with the correct designation and that a correct ladder is applied to each analysis sample set.

I. Check and confirm off-ladder alleles, making note of any samples needing re-analysis or re-run

J. Check and insure that each sample is correctly labeled with allele designation and peak height

K. Print electropherograms of all samples, controls, and ladders used in analysis

L. Save file according to CBI conventions

INTERPRETATION OF DATA

The interpretation of casework results is a matter of both professional judgement and expertise. The following objective criteria are to be used by the analysts to aid in their

interpretation of the data presented them. These criteria are based upon validation studies, literature and casework experience.

These guidelines are not a set of hard and fast rules, but rather a means to establish a general framework and outline minimum standards to insure that:

A. conclusions in the casework report are scientifically supported by the analytical data, including that obtained from appropriate standards and controls;

B. interpretations are made as objectively as possible; and

C. interpretations are consistent from analyst to analyst.

EVALUATION OF STR DATA

The goal of the evaluation and interpretation of the STR data is to determine the DNA profiles developed from the analysis of case samples and comparison of these profiles with those from known individuals. The following should be applied to aid in that interpretation.

A peak is defined as a triangular section of the electropherogram that results from the sum of input signals and as such shows Gaussian distribution.

The internal lane standard (GS-500[ROX]) must have the correct sizes assigned to the peaks used for sizing. It is noted that the 250 bp is not used for sizing purposes. The 75 and 350 bp peaks must be captured for all samples. The 400 bp peak must be captured for the D18S51 allele or other alleles greater than 340 bp in size.

The ladder used by Genotyper to call the alleles of the samples analyzed must be correctly labeled with the correct allele designations.

Genotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus.

The minimum peaks threshold must be 150 RFU or greater to be considered for match purposes. Peaks between 50 and 150 RFU will be interpreted with caution and utilized for the following.

A. If it provides exculpatory information, it can be noted on the report.

B. If it indicates the presence of a mixture, it can be noted in the report.

Homozygous allele peak heights are in general approximately twice that of heterozygous peaks as a result of doubling of the signal from two alleles of the same size. Caution should be used with the interpretation of low RFU single allele peaks. They may indicate homozygous alleles, but could also be the result of preferential amplification.

Genotypes can still be interpreted even when some loci amplify poorly. Each locus is independent of the others allowing for results to be interpreted and a genotype profile obtained at the other loci. Poor amplification at a locus may be due to degraded DNA, presence of inhibitors, extremely low or excessive input DNA, or primer mismatch.

Samples may not be re-injected for longer than 5 seconds. Peak height ratios of heterozygote alleles are defined as the percentage of the lower peak's height to the higher peak's height, expressed as a ratio. Peak heights below 70% may possibly indicate the presence of a mixture. However, a single source sample may have a peak height ratio below the 70% associated with a heterozygous allele at a given locus. Careful examination of all loci with the indication of other peak height ratios <70% and/or the presence at a given locus of three or more alleles will aid in the interpretation of the sample. Depending on the sample source, the locus in question, the number of loci affected, and the percent disparity between alleles, the sample may need to be reamplified and typed. Degraded DNA, inhibitors, low amount of target DNA, or primer mismatches may be causes for the peak height ratio imbalance. In cases where not all of the loci in the kit are developed, a partial DNA profile is obtained and so noted in the report.

Within a single source sample, genotypes generated for D3S1358 and D7S820 in the Profiler Plus and Cofiler amplification and typing systems must be concordant.

Artificial peaks within the analysis range, other than target peaks, may be detected on the electropherograms. These extra peaks may be caused by stutter, incomplete A-addition, pull-up, or spikes.

STUTTER

Stutter is predominantly a minor product peak four base pairs shorter (n-4) than the corresponding allele peak (n). It is also possible, in the case of high target DNA concentration, to see n+4 addition and n-8 stutter. These stutter peaks may be due to slippage during amplification and sequence microvariants can affect the amount of stutter. (E.g. a lower amount of stutter is produced from alleles with increased sequence variation between repeats.)

The following table values should be used as guidelines for the expected levels of stutter (measured as a % of n).

% Stutter for Profiler Plus Loci

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
% Stutter	12	12	12	10	11	14	10	9	9

% Stutter for Cofiler Loci

Locus	D3S1358	D16S539	THO1	TPOX	CSF1PO	D7S820
% Stutter	12	10	5	6	9	9

For purposes of mixture interpretation, peaks in the n-4 position that exceed the expected percent stutter at a particular locus may be designated as a true allele.

Stutter peaks may be elevated above established thresholds by the following.

A. Off-scale raw data (RFU greater than 8100). If the stutter peak is greater than the maximum allowed and the primary peak is above 3000 RFU and/or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be diluted and re-injected to resolve the issue.

B. For alleles differing by two repeats, the stutter peak from the larger allele may overlap the trailing shoulder of the smaller allele and therefore exhibit an increased stutter percentage. This will not be the case if the smaller allele goes to the baseline before reaching the stutter peak.

C. Stutter peaks overlapping an area of elevated baseline may exhibit increased percentages. The analyst may choose to apply a different matrix and re-analyze the sample with GeneScan software.

D. The presence of a minor component (as in mixture) at the same location as a stutter peak.

NON-TEMPLATE NUCLEOTIDE ADDITION (A-ADDITION)

AmpliTaq Gold® DNA Polymerase can add an additional nucleotide to the 3' ends of double stranded PCR product. This addition creates a species n+1 nucleotide in length. The n+1 species is considered a true allele. In some instances, most notably in situations of sample overload and possibly suboptimal PCR conditions, there can be incomplete addition (n) and two species will result that differ by one base pair (n and n+1). The following considerations should be made in mixture interpretation to determine if a peak in the n position is a true peak, an allele, or possibly both.

A. Determine if all the alleles are accounted for.

B. Determine at which loci the n peaks are present (vWA, D3S1358, and THO1 seem to present this situation most readily).

C. Determine if common alleles are known to exist at these loci in the n position.

Samples displaying split peak characteristics (indicates excess target DNA) may be incubated again at 60ºC (to complete the A-addition), diluted and then re-typed.

NOTE: Microvariants may differ by only one base pair so interpretation should be made with caution. Microvariants will not change base pair value upon re-amplification and retyping.

NOTE: Shoulder peaks occur at the Amelogenin locus in the n position (here n+1 = Amelogenin). These shoulders are not due to insufficient nucleotide addition of the PCR product, but rather a phenomenon of capillary electrophoresis. These peaks do not interfere with interpretation at this locus.

PULL-UP

Small artifactual peaks can appear in other colors under true peaks. This phenomenon is termed “pull-up.” Pull-up is the result of spectral overlap between the dyes, which are normally corrected for by the Matrix. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at approximately the same size as the true peak. Pull-up can occur as a result of the following:

A. application of a sub-optimal matrix and

B. amplification using excess input DNA which may lead to off-scale peaks.

SPIKES AND OTHER ANOMALIES

Transient fluorescent materials in the injection (i.e. precipitated urea, fabric dyes, air bubbles etc.) as well as electrical impulses can cause artifactual peaks. Most artifactual peaks can be shown to be false by re-injection of the sample. (The peaks fail to show in the same bp size location.) Spikes are one of the most common anomalies and can occur in one, two, three or all four colors. Artifactual peaks occurring in all four colors are the easiest to diagnose as they will be the same size and of similar height (within an order of magnitude of each other); they do not need to be re-injected if they do not interfere with the interpretation of the sample. Artifactual peaks occurring in less than four colors should be interpreted with caution (especially single color spikes) and the analyst may choose to re-inject to insure a clear interpretation. Generally spikes are thin peaks with variable peak heights, often having the same scan number.

OFF-SCALE DATA

Multicomponent analysis of off-scale data may result in raised baselines, excessive “pull-up” of one or more colors under the off-scale peaks or unnaturally high stutter peak ratios. Off-scale data may still be interpreted with caution. Samples with off-scale data (8100 RFU in unanalyzed data) may be re-injected with less time (2-3 seconds), diluted and retyped or in some cases re-amplify and type the sample using a smaller amount of target DNA.

OFF-LADDER ALLELES

Peaks not aligning with those in the allelic ladders may be detected both within and outside of the range of the allelic ladders. The Genotyper software will accurately label many of the alleles not present in the allelic ladders; however, manual genotyping may be used to determine a genotype where Genotyper does not make the assignment. Manual genotypes are assigned using the base pair calculations by the GeneScan software. Whenever an off-ladder allele is considered a true off-ladder allele (a variant allele rather than an off-ladder allele due to spikes, pull-up, split peaks etc.) it must be confirmed by re-amplification and re-typing if it conforms to the same overall guidelines described in the Interpretation of Data. During the course of analysis, if the same off-ladder allele occurs in two or more different sample amplifications, then re-amplification

and re-typing are not necessary. The existence of a microvariant is confirmed by its presence in the multiple samples.

Off-ladder alleles (OLA) that fall between alleles within the ladder will be designated in accordance with guidelines of the International Society of Forensic Haemogenetics. OLA calls are first converted to sizes in base pairs then compared to the size of the appropriate ladder alleles and the allelic designation determined. If the OLA is not a perfect repeat but rather varies by 1, 2, or 3 base pairs from a ladder allele, then it will be designated as an integer of that variation. For example, if a green OLA peak size is 238.89 and the 36 allele of D21S11 ladder is 236.32 bp, then the peak will be designated D21S11 36.2.

Alleles smaller than the ladder's lowest molecular weight allele (i.e. A) will be designated <A. Alleles greater than the ladder's largest molecular weight allele (i.e. B) will be designated >B.

Significant room temperature fluctuations may result in size variation between injections during a single run such that allelic ladder peaks differ by more than ±0.5 bp from allelic peaks in other injections. Genotyping with another allelic ladder may alleviate this problem. If not, then the cases may be broken down into sections such that a ladder associated with that case can be used to correctly type the samples associated with that case.

REAGENT BLANKS

A reagent blank tests for possible presence of contamination of the extraction reagents and/or supplies by an adventitious source of DNA. The adventitious source of DNA may be non-amplified DNA or PCR product. Peaks >50 RFU, not attributable to artifacts, located between 100 and 350 bp or at 75 bp and 400 bp indicate the presence of contamination and none of the samples extracted or amplified with the reagent blank will be considered inclusive for match purposes. If they cannot be re-amplified, then these samples can be considered for exclusion purposes. If artifactual peaks occur, then the reagent blank must be re-injected.

NEGATIVE AMPLIFICATION BLANK

A negative amplification blank is a test for the possible presence of contamination occurring during the amplification setup. If a negative control exhibits peaks >50 RFU (not attributable to artifacts), located between 100 and 350 bp or at 75 bp and 400 bp, they indicate the presence of contamination and none of the samples amplified with the negative amplification blank will be considered inclusive for match purposes. If they cannot be re-amplified, then these samples can be considered for exclusion purposes. If artifactual peaks occur, then the negative amplification blank must be re-injected.

POSITIVE EXTRACTION AND AMPLIFICATION CONTROLS

The positive control should have all the alleles present and they must be genotyped correctly. If there appears to be a problem with the injection or an electrophoretic

problem, the control and ladder can be re-injected. If the correct type is obtained, the run can be used for match purposes.

If this does not solve the problem, the entire set should be re-amplified and typed. The previous run can be considered for exclusion purposes but will be inconclusive for match purposes.

INTERPRETATION OF THE SPECIMENS

The following interpretation standards are to be used as a guide. This set of guides will cover most routine case scenarios. Cases that fall outside of these guidelines will be addressed through discussion with the Technical Reviewer, Technical Leader, and/or Agent-In-Charge, in order to achieve a reportable opinion.

SINGLE SOURCE CONTRIBUTORS

A sample can be considered to have originated from a single source if:

A. Only one or two alleles are present at all loci examined, although three peak patterns can occur, and/or

B. The peak height ratios of the heterozygotes fall within expected values.

Population statistics will be calculated for probative single source samples when allele peaks represented are greater than or equal to 150 RFU.

Locus Evaluation

Both peaks of a heterozygote must be represented and be greater than or equal to 150 RFU.

A locus can be determined to be inconclusive and a value for the frequency of that locus assigned the value of one if it is below 149 RFU. If an exclusion can be made with peaks below a value of 149 RFU then the allele can be used in evaluation.

In the event that all loci are below 150 RFU, the sample can be concentrated and re-evaluated if sample remains. If an exclusion can be made with this information it must be used.

MULTIPLE SOURCE CONTRIBUTORS

A sample can be considered to have originated from multiple (two or more) sources if:

A. More than two alleles are present at two or more loci (although three peak patterns can occur from a single source sample) and/or

B. The peak height ratios for heterozygotes fall outside the expected values when all loci are considered.

Note: Alleles with peak heights between 50 and 149 RFU can be incorporated in this assessment.

Major/Minor Contributors:

A sample can be considered to consist of a major/minor contributor mixture if there is a distinct contrast in peak intensities between alleles and the alleles contributing the largest peak height values satisfy the conditions of a single source specimen. All loci for which a result was obtained must be considered when making this determination. Interpretation of the major contributor will be restricted to those major component peaks greater than or equal to 150 RFU. Providing these conditions exist, a genotype frequency will be reported for the major contributor.

The genotype of the minor contributor can be determined if the peaks, once the major contributor's alleles have been accounted for, satisfy the conditions of a single source specimen. All loci must be considered when making this determination, and there must be a clear distinction of the minor component from the major component at all loci (all 9 if Profiler Plus, and all 13 if both Profiler Plus and Cofiler utilized). Interpretation of the minor contributor will be restricted to those minor component peaks greater than or equal to 150 RFU. Providing these conditions exist, the minor component will be considered defined and a genotype frequency will be reported for the minor contributor.

Mixtures With Known Contributor

In instances where a contributor is known or expected to be present (e.g. certain sexual assault samples), assignment of the known contributor's alleles may allow for the determination of the remaining profile and a frequency may be reported.

Indistinguishable Mixtures

If a major and minor contributor cannot be distinguished because of similarity in peak heights or the presence of overlapping alleles that cannot be assigned, individuals may or may not be included or excluded and a combined genotype frequency may be determined using the mixture calculation defined below. In this instance, all alleles greater than or equal to 50 RFU will be included in the calculations.

APPLICATION OF POPULATION FREQUENCY DATA TO PROFILER PLUS AND COFILER TYPING RESULTS

The **Allele Frequencies Appendix** lists the Profiler Plus and Cofiler STR allele frequencies for three population (African-American, Caucasians and SW Hispanics). Additionally other databases are available in the literature, if needed.

Genotype frequencies obtained for DNA alleles at one locus can be multiplied by the frequencies found for the same sample at the other twelve loci to obtain a combined frequency estimate.

Heterozygote frequencies will be calculated using the formula:

$$f=2pq.$$

Homozygous frequencies will be calculated using the NRC II formula:

$$f=p^2+P\,(1-P)\theta \text{ with } \theta= 0.01.$$

In a case where a small, isolated population (e.g. Native American) is relevant, frequency calculations for that population will use 0.03 for θ.

Note: Amelogenin is not incorporated in the population statistics.

Popstats, in the FBI CODIS system, may be utilized to obtain the statistical analysis results associated with a given DNA profile.

Minimum allele frequencies

A minimum allele frequency estimation is calculated for STR loci which demonstrate alleles with a low frequency of occurrence. The intent of this application is to set a lower limit for the frequency for such rare alleles and, consequently, produce a conservative allele frequency estimate that does not underestimate the allele's frequency of occurrence. The approach utilized is a basic procedure described previously by Budowle et al, 1991 and National Research Council, 1996. The minimum allele frequency is calculated using the following expression:

$$\mathbf{P_{MIN}} = \frac{\mathbf{5}}{\mathbf{2}n}$$

where n represents the sample size (individuals).

Mixture calculations

A mixture calculation may be performed using the allele frequencies of each allele represented in a profile with intensity greater than or equal to 50 RFU to determine the probability of selecting an unrelated individual in the population who could be a potential contributor to the mixture.

To calculate the frequency of occurrence for individual STR loci and multi-locus STR-DNA profiles when a mixture is present:

A. The allele frequency for each allele greater than or equal to 50 RFU in the mixed DNA profile for an individual locus is determined.

B. The individual fragment frequencies are summed, then this value is squared:

$$(\mathbf{P_1} + \mathbf{P_2} + \ldots \mathbf{P_n})^2 = \mathbf{P_{LOCUS}}$$

where n equals the number of alleles present, and P_{LOCUS} is the probability of an unrelated individual in the population being a contributor to that mixture at that locus.

C. Multiply each value of P_{LOCUS} to generate the probability of an unrelated individual in the population being a contributor to that mixture for all loci examined.

$$\mathbf{P_{LOCUS\ 1}} \times \mathbf{P_{LOCUS\ 2}} \cdots \times \mathbf{P_{LOCUS}}_{\ n} = \mathbf{P_{mix}}$$

Where n equals the number of loci analyzed and present in the mixture.

D. Calculate the percent of the population that can be excluded as a possible donor to the mixture of DNA in the sample as follows:

$$100 \times (1 - P_{mix}).$$

CRIMINAL PATERNITY CASES

In rare cases it may be necessary to evaluate by genetic testing a parentage of a biological substance. The Colorado Bureau of Investigation will participate in these types of cases in a limited capacity.

The genetic testing of the material will be performed according to the current Colorado Bureau of Investigation Standard Operating Procedure.

The alleles found in the child's sample are referred to as obligate alleles, that is the child must have inherited these alleles from their biological parents. If a suspected parent and a child share an obligate allele then the individual cannot be excluded as a potential biological parent. The frequency of other Random Parents Not Excluded (RPNE) in the population would then need to be calculated. This frequency, the RPNE, determines the number of random individuals that could also not be excluded as potential biological parent(s). This frequency is much the same as what is calculated for single source samples. (The question being answered is: what is the chance that a random person would also not be excluded as a potential biological parent?) The RPNE is based on the obligate alleles in the child's sample. The calculations are as follows.

If at locus 1 the child is heterozygote (has two obligate alleles) then any person with either of those obligate alleles could not be excluded as a potential biological parent. So for example if at locus 1 the child is type 8, 10 then any individual with an 8 or a 10 at locus 1 could not be excluded. Therefore the RPNE is going to equal the frequency of 8,8; 10,10; 8,10; 8,X and 10,X or

RPNE locus 1 $= p^2 + q^2 + 2pq + 2p(1-p-q) + 2q(1-p-q)$

$= 1 - (1-p-q)^2$ **equation 1**

If, on the other hand, the child is homozygote (has one obligate allele) then any person with that allele could not be excluded as a potential biological parent. So for example if at locus 2 the child is type 15, 15 then any individual with a 15 at locus 2 could not be excluded. Therefore the RPNE is going to equal the frequency of a 15,15 and 15,X or

RPNE locus 2 $= p2 + 2p(1-p)$

$= 1 - (1-p)2$ equation 2

The probability of exclusion (PE) would then become:

$PE_{locus\ 1} = 1\text{-}RPNE_{locus\ 1}$

$PE_{locus\ 2} = 1\text{-}RPNE_{locus\ 2}$

The combined PE for all loci tested would be

$PE_{combined} = 1 – (RPNE_{locus\ 1} * RPNE_{locus\ 2} *….RPNE_{locus\ n})$

The proportion of the population excluded as the possible parent(s) is expressed in report form as $100 * PE_{combined}$ in terms of a percent.

For any case in which the parents of a living or dead person are unknown, referred to as reverse parentage, the equations above do not change. Examples of cases involving reverse parentage are missing person cases involving suspected crime scenes, a single bone found in a particular location or an abandoned child.

There will be times in working criminal parentage cases that one of the biological parents will be known, i.e. a sexual assault in which a baby is conceived. In this case the mother of the child would be known and it may be possible to determine which obligate allele she passed to her child. In cases such as these it may be possible to determine specifically which obligate allele must have been passed from the biological father.

Mother	8, 9	
Child	8, 14	Obligate allele 14
	OR	
Mother	8, 9	
Child	8	Obligate allele 8
	OR	
Mother	8, 9	
Child	8, 9	Obligate alleles 8 and 9

If there is only one obligate allele, use equation 2. However, if there are two obligate alleles, use equation 1. Again once the RPNE is determined for each locus then the PE for all loci would be determined.

If the questioned parent(s) do not share the obligate allele(s) with the child then an exclusion has occurred. To determine that the parent(s) are excluded as possible biological parents there must be at least three (3) exclusions. That is the child must not share an obligate allele with the parent(s) in at least three (3) different loci. In these cases the parent(s) are excluded and no calculations are necessary.

Finally, considering cases in which there are exclusions at only one (1) or two (2) loci. The explanations for this occurring are as follows. First is that the parent(s) is not the biological parent(s) and additional exclusions will be obtained when different systems are analyzed. The second is that there has been a mutation(s). Finally, a sibling of the suspected parent is the biological parent. In these cases additional information may be necessary. Refer to your AIC for current information as to how to handle cases with exclusions at only one (1) or two (2) loci.

Kinship Relationship

In some cases it may be necessary to evaluate by genetic testing that an individual is the biological sibling or some other distant relative of someone that is the source of a stain, tissue or biological substance. These cases shall be referred to an external agency or laboratory that participates in these evaluations on a regular basis. The Colorado Bureau of Investigation will indicate no preference to which agency or laboratory is used. Performing the genetic analysis using current CBI standard procedures and reporting only the genetic profile of each sample tested can provide the assistance to the requesting agency. The requesting agency can utilize this information in soliciting statistical inference on the relationships between individuals and samples submitted.

ELECTRONIC FILE NAMING CONVENTION FOR THE 310 STR ANALYSIS

The case number forms the basis for naming electronic files. In the example below, the case number is D50-5010.

Date is listed as month/day/year for Mac and month-day-year for IBM.

ai = analyst's initials lower case
PP = Profiler Plus
CO = Cofiler

Case #	D50-5010
Sample Number	1D505010 – item # 1 from case D50-5010
Sample Number (Semen)	IE-SD505010
Sample Sheet	SS dateaiPPand/orCO
Run Folder	Run Folder dateaiPP and/or CO
GeneScan Project	GS Project dateaiPPand/or CO
Matrix file	Matrix dateaiinstrument
Size Standard	GS500dateaiPP or CO
Genotyper document edited	GT dateaiPP or CO
Genotyper document – case electropherograms	GT dateaiPP or COD50-5010 **Mac date**
Genotyper document – case tables	GT dateaiPP or COD50-5010 **IBM date**

All case data will be stored associated with the month and date of the run. That folder will be duplicated onto a write-protected CD. One CD will be stored in the lab from which the data originated and the second will be stored as follows:

Denver to Pueblo
Pueblo to Montrose
Montrose to Denver.

REPORT WRITING

CONTENTS OF PCR REPORTS

All PCR reports shall contain:

- date of submission and completion,
- laboratory case number,
- agency case number,
- name and ORI of submitting agency,
- item number and description of evidence,
- list of loci analyzed,
- summary of the test results/conclusions,
- Interpretative statement (either quantitative or qualitative),
- statement regarding disposition of evidence,
- examiner designation (initials) and page numbering, and
- signature and title of the person accepting responsibility for the content of the report.

GENERAL CATEGORIES OF TESTING CONCLUSIONS

Exclusion

The genetic information detected for the samples under comparison is different and could not have originated from a common source.

Inclusion (match)

The genetic information detected for the samples under comparison is the same and the possibility of originating from a common source cannot be excluded.

Statistical information is generally included for probative comparisons, which result in some level of inclusion.

This is typically reported as the probability, based upon genotypic frequencies, of observing a random match among unrelated individuals from the population. If, however, the most common frequency for the racial groups determined is 1,000 times greater than the population of the United States, then the following statement may be made: “To a reasonable degree of scientific certainty, ___ is the source of the DNA in item #___.

Inconclusive

Due to limited amount of information present (sometimes only a few loci examined), an inclusion statement cannot be issued regarding the comparison; however, in some instances, the results may be defined in terms of the ability or inability to exclude.

Note: In the case of mixtures, the results may be described in terms of a known sample being excluded or included as a potential major/minor contributor to the genetic material detected in a questioned stain.

POPSTATS

The CBI may use the POPSTATS software program to calculate population statistics. POPSTATS enables one to determine the statistical probability of selecting a person at random from a given population and matching the information contained in a given target profile. Alternatively, POPSTATS may be used to perform mixture calculations (Probability of Exclusion). The POPSTATS program has been distributed to forensic laboratories as part of the CODIS software.

Calculating genotype frequencies for casework DNA profiles

A. Currently, the frequency data from the FBI databases is used.

B. The unbiased expected frequency is determined directly from the allele frequencies of the population data using the CODIS POPSTATS 5.2 software program.

   1. The NRC II (1996) 4.2 Option featuring the theta value for homozygote frequencies is selected. A theta value of 0.01 is used for all populations.

   2. Refer to the *POPSTATS 5.1 Calculation Specification Manual* for all statistical formulas.

   3. A 0.01 default value is used for alleles that have not been detected within the populations or have been detected at a frequency below 0.01.

C. A POPSTATS report will be provided for all cases in which statistical calculations have been conducted. This report will give frequency data for Caucasian, African American, and southwestern Hispanic populations. This report will be kept in the case file and will be peer reviewed.

NOTE: Please refer to sections Interpretation of the Specimens on page 13 and Application of Population Frequency Data to Profiler Plus and Cofiler Typing Results on page 13 for information on statistical methods and casework interpretation and statistical references.

PRINCIPLES OF STR ANALYSIS DATABASE

The AmpFℓSTR® Profiler Plus™ (Profiler Plus or PP), AmpFℓSTR® Cofiler™ (Cofiler or CO) and AmpFℓSTR® Identifiler™ (Identifiler or ID) DNA typing systems (Applied Biosystems) utilize the polymerase chain reaction (PCR) to amplify regions of DNA known as short tandem repeats (STRs) in order to characterize DNA extracted from database specimens. Profiler Plus, Cofiler and Identifiler are multiplex systems which allow for the simultaneous amplification of numerous STR loci as well as a portion of the amelogenin gene located within the X and Y-chromosomes. Analysis of amelogenin allows for gender determination. The Profiler Plus, Cofiler and Identifiler kits contain all reagents needed for amplification, which includes: primer sets which are specific for the various loci, PCR reaction buffer and AmpliTaq Gold® DNA polymerase as well as the required allelic ladders. The primer sets contained within each kit consist of both unlabeled primers and those that are labeled with one of three (PP/CO) or four (ID) distinctive fluorescent dyes. The use of multicolor dyes permits the analysis of loci with overlapping size ranges as well as the inclusion of a sizing standard with each sample. The amplified fragments are separated according to size by electrophoresis using the ABI Prism™ 377 DNA Sequencer or ABI Prism™3100 Genetic Analyzer. The fluorescent dyes labeling the amplified fragments are detected by laser excitation and a charge-coupled device (CCD) camera. The resulting data is graphically displayed as colored peaks noted by height in relative fluorescent units (RFUs) and time (scan number). This display is called an electropherogram.

The reference allelic ladders for each of the STR loci and reference fragments for amelogenin also undergo electrophoresis. These allelic ladders contain the more common alleles in the general population for each locus. Using the ladders, the alleles present in DNA samples may be determined.

The following tables, Table 5 and Table 6, list the Profiler Plus, Cofiler and Identifiler loci, the size ranges of alleles within a particular locus, the alleles present in the ladder, the color of the labeled primer and the DNA profile of the positive control (9947A). Note that some of the same loci are amplified in both the Profiler Plus and Cofiler.

Additional information, procedures, and protocols may be relied on from the *ABI PRISM 377 DNA Sequencer User Manual* (1998), *ABI 373 and ABI PRISM 377 DNA Sequencers GeneScan Reference Guide* (1997), the *ABI PRISM 3100 Genetic Analyzer User Manual* (2001), *GeneAmp PCR System 9700 User's Manual Set (1997),* the *AmpFℓSTR Profiler Plus™ User's Manual* (1998**)** , the *AmpFℓSTR Cofiler™ User's Manual (1998),* and the *AmpFℓSTR® Identifiler™ User's Manual* (2001).

Table 5

SYSTEM	LOCUS	SIZE RANGE (bp)#	ALLELES PRESENT IN LADDER[1]	DYE COLOR	POSITIVE CONTROL 9947A
Both	D3S1358	114-143	12 - 19	FAM-5 Blue	14, 15
Profiler Plus	vWA	157-197	11 - 21	FAM-5 Blue	17, 18
Profiler Plus	FGA	220-269	18-26, 26.2, 27-30	FAM-5 Blue	23, 24
Both	Amelogenin	106, 112	X, Y	JOE Green	X, X
Profiler Plus	D8S1179	127-172	8 - 19	JOE Green	13, 13
Profiler Plus	D21S11	189-244	24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 38	JOE Green	30, 30
Profiler Plus	D18S51	274-343	9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-26	JOE Green	15, 19
Profiler Plus	D5S818	134-171	7 - 16	NED Yellow	11, 11
Profiler Plus	D13S317	207-236	8 – 15	NED Yellow	11, 11
Both	D7S280	259-294	6 – 15	NED Yellow	10, 11
Cofiler	TH01	169-190	5-9, 9.3, 10	JOE Green	8, 9.3
Cofiler	TP0X	218-247	6 – 13	JOE Green	8, 8
Cofiler	CSF1P0	281-317	6 – 15	JOE Green	10, 12
Cofiler	D16S539	233-273	5, 8-15	FAM-5 Blue	11, 12

*Sizes are actual base pair size of alleles in the Profiler Plus and Cofiler ladders (including the nucleotide addition). The sizes obtained in Genotyper may vary from the actual fragment sizes due to electrophoretic effects.

[1] The allelic ladders provided in the kit are used to determine the genotypes of the samples.

Table 6

SYSTEM	LOCUS	SIZE RANGE (bp)*	ALLELES PRESENT IN LADDER[1]	DYE COLOR	POSITIVE CONTROL 9947A
Identifiler	D8S1179	123-170	8-19	6-FAM Blue	13,13
	D21S11	185-240	24,24.2,25-28,28.2,29,29.2,30, 30.2,31,31.2,32,32.2,33,33.2,34,34.2,35, 35.2,36-38	6-FAM Blue	30,30
	D7S820	255-292	6-15	6-FAM Blue	10,11
	CSF1PO	305-342	6-15	6-FAM Blue	10,12
	D3S1358	112-140	12-19	VIC Green	14,15
	THO1	163-202	4-9, 9.3,10,11,13.3	VIC Green	8,9.3
	D13S317	217-245	8-15	VIC Green	11,11
	D16S539	252-293	5,8-15	VIC Green	11,12
	D2S1338	307-359	15-28	VIC Green	19,23
	D19S433	102-135	9-12,12.2,13,13.2,14, 14.2,15,15.2,16,16.2,17,17.2	NED Yellow	14,15

Table 6

SYSTEM	LOCUS	SIZE RANGE (bp)*	ALLELES PRESENT IN LADDER[1]	DYE COLOR	POSITIVE CONTROL 9947A
Identifiler	vWA	155-207	11-24	NED Yellow	17,18
	TP0X	222-250	6-13	NED Yellow	8,8
	D18S51	262-345	7,9,10,10.2,11-13, 13.2,14,14.2,15-27	NED Yellow	15,19
	Amelogenin	107-113	X,Y	PET Red	X,X
	D5S818	134-172	7-16	PET Red	11,11
	FGA	215-355	17-26,26.2,27-30, 30.2, 31.2,32.2,33.2, 42.2,43.2,44.2,45.2, 46.2, 47.2,48.2,50.2, 51.2	PET Red	23,24

*Sizes are actual base pair size of alleles in the Identifiler ladder. The sizes obtained in Genotyper may vary from the actual fragment sizes due to electrophoretic effects.

The allelic ladders provided in the kit are used to determine the genotypes of the sample

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[1] The allelic ladders provided in the kit are used to determine the genotypes of the sample

GUIDELINES FOR CONTROL SAMPLES

It is imperative that proper control samples be included when database samples are extracted, quantified (where appropriate), amplified, and typed. The typing results obtained from these control samples are essential for the interpretation of STR and amelogenin typing results from database samples. The controls also serve to assess the quantity and quality of the extracted DNA as well as the effectiveness, accuracy, and precision of the analytical procedures.

EXTRACTION CONTROLS

Reagent Blank

With each database extraction set of samples a reagent blank (RB) must be included. The reagent blank consists of all reagents used in the test process minus any sample. The reagent blank is processed through the entire extraction, quantification (where appropriate), amplification, and electrophoretic typing procedure. (With FTA extractions, the reagent blank tube is subjected to the same extraction conditions as the samples.) The reagent blank must be run in all PCR systems to check for contamination of the reagents with extraneous DNA.

AMPLIFICATION CONTROLS

Negative Amplification Blank

A negative amplification blank (NAB) must be used with each amplification. The negative amplification blank contains all components required for the amplification of DNA except that no DNA is added. TE Buffer is placed in the negative amplification blank in lieu of a DNA solution. The negative amplification blank is processed through the amplification and electrophoretic typing procedures.

Positive Amplification Controls

DNA from cell line 9947A is provided with the reagent kits. This is used as a positive control for STRs and amelogenin to evaluate the performance of amplification and electrophoresis. When the control specimen 9947A is amplified, the STR loci must solely exhibit the correct genotype. Additionally, specimen 9947A is the female control for amelogenin and must exhibit a single band at the position corresponding with the size ladder band representative of the 106 bp peak from the X chromosome.

According to the AmpFlSTR Identifiler PCR Amplification Kit User's Manual, an appropriate cycle number for the high quantity of DNA obtained from a 1.2 mm bloodstained FTA punch is 25 cycles. Because the positive amplification control (9947A) is not sufficiently amplified in the suggested 25 cycles, a NIST traceable CBI internal control consisting of a blood standard of a known type spotted onto FTA paper can serve as the positive amplification control. This internal control may differ for each laboratory. The genotype for the CBI internal DNA controls is kept in the logbook with the results of the kit validations.

EQUIPMENT

DNA THERMAL CYCLER 480 AND 9700

The DNA Thermal Cycler 480 or 9700 can be used to amplify both Chelex™ extracted and FTA extracted database samples. The function of the thermal cycler is critical to the proper performance of the STR typing procedure; thus, the thermal cycler is calibrated semiannually. See the User's Manual for each model instrument for further details on calibration.

ABI PRISM™ 377 DNA SEQUENCER

The ABI Prism™ 377 DNA Sequencer is a fluorescent gel imaging system that is capable of determining base pair sequence, fragment size, or relative quantity of fluorescent dye-labeled DNA nucleotide fragments. It consists of an electrophoresis instrument with a laser excitation and CCD camera imaging system and a desktop computer with software for data collection and analysis. Accessories such as gel cassettes, glass plates, and buffer reservoirs are included. See the *ABI Prism 377 Sequencer User's Manual* for further details.

ABI PRISM™ 3100 GENETIC ANALYZER

The ABI PRISM™ 3100 genetic analyzer is an automated capillary array electrophoresis system. This system can simultaneously separate, detect and analyze fluorescent labeled DNA fragments from 16 capillaries in one run. The system includes an ABI PRISM™ 3100 Genetic Analyzer and software, computer workstation with Microsoft™ Windows NT operating system, collection software, capillary array, and reagent consumables.

DESKTOP COMPUTERS

Desktop computers in the amplification room are used to run the instrumentation and collect data. Desktop computers in office areas contain software used to analyze data and obtain DNA profiles for entry into the CODIS database.

QUALITY CONTROL OF CRITICAL REAGENTS

For database analysis, critical reagents should be checked against materials known to give an acceptable result. The quality control requirements for these items are given below.

PROFILER PLUS, COFILER AND IDENTIFILER KITS

Each lot of kits will be verified using a NIST traceable CBI internal DNA control. The criterion for kit quality sufficient for use in database analysis is that a correct genotype must be obtained for the CBI internal DNA control used. Kit lots need not be verified prior to database analysis.

FTA PAPER

New lots of FTA paper should be verified prior to use on any database sample. To verify the new lot a sample is spotted on stain cards from both the new and old lots. Identical profiles must be obtained through PCR profiling. Peak heights and peak resolution should be similar. If this does not occur, a different lot of FTA paper should be obtained.

EXTRACTION PROCEDURE

Database samples are extracted with FTA and Chelex procedures. See Inorganic and FTA extraction procedures in this SOP for details.

QUANTIFICATION PROCEDURE

For samples extracted with Chelex™ an estimate of the DNA concentration of the samples will be conducted prior to STR analysis. CBI uses the QuantiBlot™ biotin-streptavidin-HRP method to evaluate the DNA concentration of each extracted sample. In general, 0.75 to 2.0 ng of DNA is optimal for STR amplification, although results may be obtained outside of this range. See the ***QUANTIBLOT® section*** of this SOP for more details.

The FTA paper extraction procedure does not require quantification.

AMPLIFICATION

All PCR setup steps must be performed in the DNA extraction/PCR setup room of the laboratory using hoods, reagents, and pipettors dedicated to this area.

The reagents required for the amplification of the thirteen core STR loci are included in two kits, the AmpF□STR® Profiler Plus™ PCR Amplification Kit and the AmpF□STR® Cofiler™ PCR Amplification Kit or in a single combined kit, the AmpF□STR® Identifiler Amplification Kit. For each amplification, combine DNA template, PCR reaction mix, Primer set (Profiler Plus, Cofiler, Identifiler) and DNA polymerase (AmpliTaq Gold®) in a reaction tube and subject this mixture to a series of controlled temperature changes in the thermal cycler.

The AmpF□STR® Identifiler Amplification Kit contains primers for two additional loci, D2S1338 and D19S433.

THERMAL CYCLER PARAMETERS

The following parameters are used to amplify the AmpF□STR Profiler Plus, Cofiler, and Identifiler loci in the thermal cyclers.

Turn on the thermal cycler and allow the instrument to warm up for a minimum of 1 hour. If using the PE 480, add one drop of oil into each of the wells to be used. Select the file for forensic STR analysis. The parameters are listed below.

DNA THERMAL CYCLER 480 OR 9700

Initial Incubation (1 cycle):	95°C	11 minutes
Cycle (step cycle file, 25 or 28 cycles*):	94°C	1 minute
	59°C	1 minute
	72°C	1 minute
Final extension (1 cycle)	60°C	90 minutes
Hold temperature (soak file)	10°C	indefinite

Tubes can be left in the thermal cycler at 4-10°C until removed. The amplified samples can then be stored at 2-6°C for a week, or longer at -15 to -20°C.

*Blood spotted on FTA paper is typically amplified at 25 cycles (this cycle number is determined through validation and may be dependent on the lot of FTA paper). Samples spotted on other substrates are typically amplified at 28 cycles.

Some samples, due to their age and condition, may require an extra final extension step of 30-45 minutes to reduce –A typing.

AMPLIFICATION MIX FORMULATIONS

The following formulation applies to both the Profiler Plus and Cofiler kits.

A. Vortex the PCR reaction mix, primer set and AmpliTaq Gold® DNA polymerase supplied in the kit for 5 seconds at medium speed. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

B. In a sterile microcentrifuge tube add:

   1. (Number of samples) x 21.0 μl of PCR reaction mix.
   2. (Number of samples) x 1.0 μl of AmpliTaq Gold DNA polymerase
   3. (Number of samples) x 11.0 μl of primer set

C. The above formulation provides a slight overfill to allow for volume lost in pipetting. The maximum volume of Master Mix held in a 1.5 ml tube can be dispensed into 42 PCR tubes. A 2.0 ml tube is recommended when preparing Master Mix for up to 55 samples. Be sure to include enough Master Mix for a reagent blank, a positive amplification control, and a negative amplification blank.

D. Mix thoroughly by vortexing at medium speed for 5 seconds.

E. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.

F. Sample preparation is detailed below. Final amplification volume is 50 μl.

The following formulation applies to the Identifiler kit:

G. Vortex the PCR reaction mix, primer set and AmpliTaq Gold® DNA polymerase supplied in the kit for 5 seconds at medium speed. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

H. In a sterile microcentrifuge tube combine:

1. (Number of samples + 1) x 10.5 μL of PCR reaction mix
2. (Number of samples + 1) x 0.5 μL of AmpliTaq Gold® DNA polymerase and
3. (Number of samples +1) x 5.5 μL of primer set.

I. The above formulation provides a slight overfill to allow for volume lost in pipetting. Be sure to include enough Master Mix for a positive amplification control, a reagent blank, and a negative amplification blank.

J. Mix thoroughly by vortexing at medium speed for 5 seconds.

K. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.

L. Sample preparation is detailed below. Final amplification volume is 25 μL.

SAMPLE PREPARATION – CHELEX™ SAMPLES USING COFILER AND PROFILER PLUS KITS

A. Prepare a DNA/TE dilution of each sample. The final amount of input DNA is 0.5 to 2 ng diluted in TE to a final volume of 20μL.

B. Determine the number of samples to be amplified. Place the required number of amplification reaction tubes into a rack, including tubes for the positive control, reagent blank and negative amplification blank. Treat the tubes with ultraviolet light for at least 20 minutes.

C. Prepare Master Mix according to Formulation instructions.

D. Dispense 30 μl of Master Mix into each reaction tube.

E. If using the 480 Thermal Cycler, add one drop of mineral oil (supplied with kit) to each tube.

F. Open each sample tube, add 20 μl of the appropriate DNA/TE sample preparation, and then close the tube. Make sure to have only one tube open at a time. Do not mix or vortex the tube.

G. Add 20 μl of the reagent blank to the tube for the reagent blank control.

H. Add 20 μl of the control DNA provided in the kit to the tube for the positive control.

I. Add 20 μl TE Buffer to the tube for the negative amplification blank.

SAMPLE PREPARATION – CHELEX™ SAMPLES USING IDENTIFILER KIT

A. Prepare a DNA/TE dilution of each sample. The final amount of input DNA is 0.5 to 1.25 ng diluted in TE to a final volume of 10 μL.

B. Determine the number of samples to be amplified. Place the required number of amplification reaction tubes into a rack, including tubes for the positive control, reagent blank and negative amplification blank. Treat the tubes with ultraviolet light for at least 20 minutes.

C. Prepare Master Mix according to Formulation instructions.

D. Dispense 15 μl of Master Mix into each reaction tube.

E. If using the 480 Thermal Cycler, add one drop of mineral oil to each tube.

F. Open each sample tube, add 10 μl of the appropriate DNA/TE sample preparation, and then close the tube. Make sure to have only one tube open at a time. Do not mix or vortex the tube.

G. Add 10 μl of the reagent blank to the tube for the reagent blank control.

H. Add 10 μl of the control DNA provided in the kit to the tube for the positive control.

I. Add 10 μl TE Buffer to the tube for the negative amplification blank.

SAMPLE PREPARATION – FTA WASHED SAMPLES FOR FTA AND S&S PAPER SAMPLES USING COFILER AND PROFILER PLUS KITS

A. Add 20 μl TE Buffer to each database sample tube.

B. Dispense 30 μl of Master Mix into each reaction tube, including those for the positive control, reagent blank, and negative amplification blank.

C. If using the 480 Thermal Cycler, add one drop of mineral oil (supplied with kit) to each of the tubes.

D. Add 20 μl of the positive control to the positive control tube; add 20 μl of TE Buffer to the reagent blank and negative amplification blank tubes.

E. If using the 9700 Thermal Cycler you may centrifuge the tubes briefly.

F. The samples are now ready for amplification.

SAMPLE PREPARATION – FTA WASHED SAMPLE FOR FTA AND S&S PAPER SAMPLES USING IDENTIFILER KIT

A. Add 10 μl TE Buffer to each database sample tube.

B. Dispense 15 μl of Master Mix into each reaction tube, including those for the positive control, reagent blank, and negative amplification blank.

C. Add 10 μl of the positive control to the positive control tube for a 28-cycle PCR; add 10 μl of TE Buffer to the reagent blank and negative amplification blank tubes. When using a NIST traceable CBI internal control as the positive control, add 10 μl TE to the tube.

D. If using the 9700 Thermal Cycler, centrifuge the tubes briefly if necessary.

E. The samples are now ready for amplification.

AMPLIFICATION

A. Place the PCR tubes in the thermal cycler and start the program. If using the 480 Thermal Cycler, record the heat block position of each tube. This is not necessary when using the 9700 Thermal Cycler.

B. After the amplification process, remove the tubes from the thermal cycler and store the amplified products protected from light. The tubes may be centifuged to get the PCR product to the bottom.

C. The amplified products can be stored at 2-6°C for no more than 1 week. For longer periods, store the tubes at -15 to -20ºC.

STR TYPING BY POLYACRYLAMIDE GEL ELECTROPHORESIS USING THE ABI 377

POURING A POLYACRYLAMIDE GEL

A 36 cm polyacrylamide gel should be poured a minimum of 2 hours prior to setting up the 377 DNA Sequencer. Gels may be stored wrapped at room temperature up to 24 hours. The following procedure describes pouring a 36 cm Long Ranger gel (Long Ranger Singel Packs, 377-36 cm WTR, product #50691, BioWhittaker Molecular Applications, purchased from Intermountain Scientific) using an Otter apparatus (also available from Intermountain Scientific).

NOTE: Orientation of the plate with respect to the gel must be consistent from gel to gel. The product number and serial number etched onto each plate must be on the outside of the "sandwich."

A. For each gel, place a clean, notched glass plate with the inside face up on the Otter. Wet two spacers and lay them on top of the plate, flush with the outside edge of the plate. Place a clean, rabbit ear glass plate inside face down at the bottom end of the notched plate.

B. Prepare pre-packaged Long Ranger gel solution by following the instructions on the gel pack. Two gels can be poured from one Singel pack.

C. Make 1500 ml 1X TBE by mixing 150 ml 10X TBE with 1350 ml ultrapure water.

D. Once the gel solution is prepared, squeeze it into a glass beaker. Then draw the solution up into a 60 ml syringe. Pour the gel by applying the solution between the plates and gradually sliding the top plate along the bottom plate, until it reaches the end. Make sure that the two plates are even and that the spacers are positioned properly. Insert the casting comb in the well region. Place clamps along the sides of the glass plates and along the comb. DO NOT place clamps along the bottom of the glass plates. A second gel may be poured at this time.

E. After 15 minutes wrap the bottom end of the gel with 1X TBE wetted Kimwipes and plastic wrap. Cover the remaining 1X TBE with plastic wrap. For overnight storage,

wrap the gel completely with plastic wrap after it has polymerized for a minimum of 1 hour.

MACINTOSH PLATFORM PROCEDURE

WARNING!! CHEMICAL HAZARD!! Formamide is an irritant and a teratogen. Avoid skin contact and inhalation. Use in a well-ventilated area. Wear lab coat and gloves when handling.

A. Turn on the ABI PRISM™ 377 DNA Sequencer.

B. Start the computer. If the computer was left on from a previous run, restart it. Launch the ABI 377XL Collection software if it is not already open.

C. Remove deionized formamide from the freezer to thaw.

D. Prepare a sample sheet (this can be done in the DNA extraction/PCR setup room and transferred over the network). The Macintosh should be restarted after the transfer.

1. Choose NEW from the File menu in 377XL Collection software.
2. Click on the GeneScan Sample Sheet icon.
3. Fill in the sample name column first. **The sample name is the PCR number followed in sequence by the CBI case number** (i.e. PCR##ZYR####). When complete, highlight the Sample Name column by clicking once in the gray bar at the top and select Copy from the Edit menu.
4. Highlight the Sample Info column and select Paste from the Edit menu.

   Note: Under the Sample Info column check that each sample has a unique sample number. Also check that each lane that contains an allelic ladder has the word "ladder" in the Sample Info column; this is necessary for automated allele calling with Genotyper (v. 2.0 or higher).

5. Check that there is a diamond next to the red box in the Std column for each sample.
6. Make sure there is an X in each box in the PRES column for the dye colors to indicate which dye colors are to be collected.
7. Check that the Std column designates red.
8. Under the Sample Info column check that each sample has a unique sample number. Also check that each lane that contains an allelic ladder has the word "ladder" in the Sample Info column; this is necessary for automated allele calling with Genotyper (v. 2.0 or higher).
9. Select Save As from the File menu and label the sample sheet.

E. Prepare notes sheet. Include the following information as a minimum:

- Analyst
- Date
- Instrument
- Long Ranger lot #
- 10X TBE lot #
- Formamide lot #
- Amplification date
- Sample dilutions
- Control dilutions
- Load volume

F. Prepare the samples for analysis.

1. Remove the GeneScan ROX 500XL (ROX 500XL) internal lane standard and allelic ladders from the refrigerator. Vortex and spin.
2. Prepare formamide loading solution (FLS) by combining 500 µl of formamide with 100 µl blue dextran (75 mg/ml 25mM EDTA). Mix by vortexing.
3. Prepare FLS/ROX by adding ROX 500XL to the FLS at a concentration necessary to obtain an RFU value of 50 to 1000 in the reagent blank and negative amplification blank. The concentration of ROX 500XL can vary depending on lot and instrument sensitivity. Make a sufficient quantity for all samples.
4. Mix the tube by vortexing.
5. Place the appropriate number of 0.5 ml tubes in a tube rack and label appropriately.
6. Add to each tube the required amount of FLS/ROX mixture, then cap all tubes.
7. Add the required amount of amplified DNA or control sample to each tube. Sample dilution for loading is dependent on extraction method, comb used, individual sample and instrument. The proper dilution needs to be determined by each analyst as a part of his or her validation studies.
8. A minimum of two ladders should be run on each gel.
9. Vortex and spin each sample tube.

The remaining sample preparations steps should be performed during the PreRun (Step J ***below).***

10. Denature samples at 95°C for a minimum of 5 minutes and no longer than 10 minutes.

11. Snap cool on ice-water bath immediately. Samples must remain on ice until loading.

G. Choose NEW from the File menu of the 377XL Collection software. Click on GeneScan Run. Import a sample sheet by clicking on the arrows in the Sample Sheet box and selecting the appropriate sample sheet. Set Auto-Analysis parameters. Check that comb type and number of lanes settings are correct.

H. Set up the gel on the instrument.

1. Place the lower buffer chamber on the 377 and plug in the electrode.
2. Remove the clamps from the gel.
3. Thoroughly clean excess acrylamide from the glass plates using ultrapure water and Kimwipes.
4. Carefully remove the casting comb and clean the comb area. Remove all excess acrylamide.
5. Insert a single use sharkstooth comb approximately 1/8" into the acrylamide.
6. Using a 1000 μl pipettor, add ultrapure water to the wells.
7. Place the plate into the gel cassette.
8. Mount the cassette onto the 377 DNA Sequencer.

I. Run the Plate Check module. If all 4 colors show a flat line, the plate is sufficiently clean. If spikes appear, remove the plate, clean the read region again with ultrapure water and Kimwipes, then repeat the Plate Check module.

J. Pre Run the gel.

1. Mount the upper buffer chamber onto the glass plates and add 1X TBE to the fill line.
2. Rinse the wells with 1X TBE. (A small amount of Formamide Loading Solution may be placed in the wells to make them visible.) Place the cover on the buffer chamber.
3. Fill the lower buffer reservoir with 1X TBE.
4. Attach the front heat transfer plate and plug in the ground wire.
5. Plug in the upper buffer chamber electrode, close the door, and start the Pre Run module.
6. Pre Run the gel. Once the temperature reaches 50ºC, samples may be loaded.

K. Load the gel.

1. Click on the Pause button. Once the instrument pauses, open the door, unplug the upper buffer chamber, and remove the lid. Rinse the wells with 1X TBE.
2. Load the samples using flat gel loading pipette tips. The amount loaded per well is dependent upon the comb used, and is determined empirically. Load all odd wells, in sequence.
3. Click on the resume button and Pre Run for 3 minutes.
4. Click on the Pause button to pause the Pre Run. Rinse the wells with 1X TBE. Load all even wells, in sequence.

L. Run the gel.

1. Terminate the Pre Run.
2. Click on the Run module, and enter a file name. Start the run.

M. At the end of the run, save the Run Folder with the date, analyst's initials and PP or CO (e.g. Run Folder mm/dd/yyanaPP/CO). Transfer the Run Folder to an analysis Macintosh for review.

N. Clean the 377.

1. Open the door to the instrument. Remove the heat plate.
2. Remove the lid to the upper buffer chamber. Rinse the lid with ultrapure water.
3. Suction out the buffer from the upper and lower buffer chambers. Use separate devices to avoid contaminating the upper buffer chamber with excess fluorescent dyes.
4. Remove the upper buffer chamber and rinse it with ultrapure water.
5. Remove the gel cassette. Unclamp the glass plates. Rinse the gel cassette with hot tap water followed by ultrapure water.
6. Disassemble the glass plates. Lay a Kimwipe across the plate to remove the gel. Soak the glass plates in hot tap water to remove any remaining acrylamide. Rinse them thoroughly with hot tap water followed by ultrapure water and allow them to air dry in a plate rack. Rinse the spacers in hot water followed by ultrapure water. USE ONLY WATER TO CLEAN THE GLASS PLATES. DETERGENT OR ETHANOL MAY CONTAIN FLUORESCENT PARTICLES THAT COULD ADHERE TO THE GLASS AND CAUSE BACKGROUND FLUORESCENCE.

7. Check the antifreeze bottle on the right side of the instrument. It should be at least 2/3 full. If necessary, replenish with 5% antifreeze diluted in ultrapure water.

STR TYPING BY CAPILLARY GEL ELECTROPHORESIS USING THE ABI PRISM 3100 GENETIC ANALYZER

A. Start the computer. Make sure the Orbixweb™ software has launched before proceeding.

B. Turn on the ABI PRISM™ 3100 Genetic Analyzer. Wait until the green light on the 3100 is on before proceeding. (See Chapter 3 in the ABI PRISM™ 3100 Genetic Analyzer User's Manual under Starting the 3100 System for more information.).

C. Check the hard drive to make sure there is enough room on the computer for sample data by running the Diskspace Utility (D:\AppliedBio\3100\Bin). If there is not enough computer memory space you may have to run Cleanup DataBase (D:\appliedBio\3100\Bin) Utility. (Refer to the procedure in the ABI PRISM™ 3100 Genetic Analyzer User's Manual, Chapter 7.) Reminder: this utility will delete all run data and plate records from the database. However, it will not affect spatial and spectral information.

D. Launch the 3100 collection software.

E. Remove the POP-4™ from the refrigerator to warm to room temperature. If crystallized urea is present when the bottle is removed, warming to room temperature and gentle mixing (rotate slowly and carefully by hand to avoid introducing air into the POP-4™) will dissolve the urea. If the precipitate will not dissolve, do not use the POP-4™. Check that the POP-4™ is specifically for use with the 3100.

F. Set up the instrument.

1. Prime the syringes. Draw up filter-purified water into the syringes and then expel. Draw up approximately 0.2 ml of room temperature POP-4™ into the reserve syringe. Pull plunger so that the tip is drawn to the position where the polymer will be filled. Remove air bubbles from the tip and slowly expel the POP-4™ onto a Kimwipe or waste container. Follow the same procedure with the capillary array syringe, using approximately 0.1 ml of POP-4™.

2. Fill both the reserve syringe and the capillary array syringe with room temperature POP-4™. Try to minimize the amount of air bubbles in the syringes. The polymer is good at 25^{o}C for 7 days, and should be changed weekly.

3. The approximate amount of polymer needed for a run can be calculated by taking the #of injections x 50-80 µl POP-4™ + 200 µl POP-4™, which is required to fill the blocks and tubing

4. Clean the outside of the syringe with distilled water then dry with a Kimwipe to remove excess polymer.

5. Remove any air bubbles by inverting the syringe and pushing out a small amount of polymer.

NOTE: It is critical that large air bubbles be expelled from the syringe. Make sure the plunger is not resting on a bed of air, as this may cause problems in the run.

6. Before installing the capillary array, launch the "Install Capillary Array" wizard from the Tools menu on the 3100 collection software. Follow the directions given in the wizard to replace or install an array. Install the array and the syringes filled with polymer. After a few times going through the wizard it may not be necessary to launch the wizard every time you reinstall an array. However, if you are replacing an array with a new one, make sure you run the wizard to log the serial number of the new array.

Note: It is not necessary to change the capillary array for every run. A capillary array should last a minimum of 100 runs. The maximum # of injections per capillary is approximately 300.

7. Fill buffer and water reservoirs. Note that the 1X buffer and DI water should be changed daily or before each run. The 1X buffer is made from the 10X buffer purchased from ABI for use on the 3100 and 310 instruments for fragment analysis.

NOTE: A larger volume of 1X buffer can be made if desired. The 1X solution can be stored at 4ºC for up to 1 month.

8. Place the 1X buffer reservoir in the #1 position (cathode buffer) on the tray rack, and water reservoirs in the #2 and #4 positions. Fill the anode buffer reservoir with 1X buffer to the red line and place it onto the lower polymer block.

9. Perform a spatial calibration. This should be performed each time the capillary array window is moved from its position. Under the "Tools" menu select "Perform Spatial Calibration." If the capillaries are not yet filled with polymer, this may be done under this menu by clicking the Fill Capillaries box. (It is not necessary to fill the capillaries each time a spatial calibration is performed.) Click the "Start" button. When the first spatial calibration is finished, click "Cancel". Per Applied Biosystems instructions, the first spatial should not be accepted. Perform another spatial calibration, clicking off the

"Fill Capillaries" function if you have already filled the capillaries during the first spatial calibration. When the second spatial calibration is finished, click the details button to view the spatial. If the spatial does not provide an adequate display, peaks are not symmetrical, or spacing between capillaries is off, rerun the spatial calibration again. When the spatial calibration is successful, click OK to accept and save this spatial. The saved spatial will be applied to all future runs until a new spatial calibration is performed. (For additional information regarding the procedure for a spatial calibration refer, to the ABI PRISM™ 3100 Genetic Analyzer User's Manual.)

10. Perform a spectral calibration if this has not been done. Spectral calibrations should be performed when the instrument is being installed, has recently been serviced in an area that affects the laser or CCD camera optics, or if the baseline in the data cannot be corrected by the current spectral calibration. Follow the procedure in the ABI PRISM™ 3100 Genetic Analyzer User's Manual in Chapter 4. Note that different spectral calibrations are used and are determined by the type of genetic profiling kit used in the analysis.

G. Plates and plate records

There are six methods for creating plate records defined in the ABI 3100 User's Manual. The Colorado Bureau of Investigation DNA Database Section has created an Excel/text format import file of a 96-well plate layout called 3100 Load Sheet and Plate Record. The plate record created is exported as a tab delimited text file format and is imported onto the 3100 Collection software as such. The plate layout may be printed out for use in loading the PCR product into the 96-well plate. It is saved as an Excel file. An additional option is to create a plate record on the instrument using the 3100 collection software. The plate record has several fields that must be filled: Well Position, Sample Name, Dye Set, Color Number, Standard Dye, Color Info, Color Comment, BioLIMS Project, Run Module, and Analysis Module. The following procedure applies to both methods:

1. Fill in the "Sample Name" and "Color Info" sections with the CBI sample name. Double check the CBI sample names when completed because this number will be used to import the sample into the CODIS database.

NOTE: ***Refer to the*** Naming Convention for STR Analysis of Database Samples section ***for the sample name format. Insure that the information contained under the Sample Name column is copied onto the Sample Info column.***

2. Fill in the plate record according to the well number where you want to place the samples. Make sure each set of 16 samples has at least one ladder. Be sure that all colors that correspond to the fluorescent labels in the PCR kit used in the amplification process are present in the "Color Number" column.

Color Comment may be left blank, but filling in this field may prevent column shifting during the plate import process.

Note: Under the Sample Info column insure that each sample has a unique sample number, also insure that each well which contains an allelic ladder has the word "ladder" in the Sample Info column; this is necessary for automated allele calling with Genotyper (v. 2.0 or higher)

3. Select a Dye Set that corresponds to the PCR amplification kit used to amplify the sample. Dye Set G5 is used for Identifiler, and Dye Set F is used for Cofiler and Profiler Plus kits.
4. Select "3100 Project 1" under the "BioLIMS project" column.
5. Select a Run Module. Genescan Run Modules are determined and created through individual laboratory validation studies run prior to DNA database analysis. Each laboratory will use the Run Module shown to work best in that laboratory. The Run Module denotes the capillary length, polymer used in collection, and the injection time.
6. Select an Analysis Module. "GS500Analysis.gsp" is typically used for fragment analysis using a 500 basepair internal size standard (LIZ or ROX). To inject a set of 16 wells more than one time, fill in more than one Run Module column and its corresponding Analysis Module. Any unused wells may be filled with internal size standard and formamide and labeled as such. Any set of 16 wells not used should be deleted from the plate record.
7. Name the plate record. The plate record name is how the 3100 collection software will recognize the plate and the information regarding the samples on that plate. The name of the plate should include the date, analyst initials, and amplification kit used (i.e.: mmddyyanaID). If more than one plate is run on a single date then, after the date, the letter A or B may be used to designate the plate location on the 3100 instrument (i.e. mmddyyAanaID for plate in the A position).
8. Save the plate record. The method used to create the plate record will determine how to save the plate record. If it was created using the 3100 collection software, clicking the OK button will save the plate and bring it up in the software as pending. If using the "Load Sheet and Plate Record" Excel format save the 3100 load sheet as a tab delimited text file and save the Excel worksheet separately.
9. Import the plate record. Import the plate record into the 3100 collection software and check to make sure that it has been imported correctly. Do this by highlighting the plate record and clicking the edit button. Make sure the columns are all filled in and that the injection number is correct. (Refer to

Chapter 6 of the ABI 3100 User's Manual for more information regarding plate records.)

H. Sample preparation. Through instrument and PCR kit validation studies, each laboratory has evaluated their own methods and DNA quantities used in sample preparation. This includes the amount of formamide, ROX or LIZ, and PCR product placed into each well on the 3100 optical plates. Each well should contain a minimum of 10ul of the combined components. After all samples are loaded onto the well plates, the samples are covered with a 96-well plate septa gasket.

1. Remove deionized formamide from the freezer to thaw.
2. Add the internal size standard to the deionized formamide and pipet this mixture into the wells of the 96-well plate.
3. Pipet the PCR products into the well determined by the plate record, making sure to include one ladder per set of 16 wells.
4. Secure the septa to the plate.
5. Denature the samples at 95ºC for a minimum of 3 minutes, and no longer than 5 minutes. (Place the entire 96-well plate into a 9700 programmed at 95ºC.)

NOTE: Septa or Septa Strips melt at high temperatures. Do not autoclave or re-use septa.

6. Snap cool on ice bath immediately for a minimum of 3 minutes.

I. Starting the run.

1. Remove the plate from the ice bath and dry off any excess moisture from the plate prior to placement into the plate rack assembly. Place the plate into the plate assembly. Make sure the plate is centered on the rack by aligning holes on the upper assembly with the holes in the septa. The assembly "clicks" together to assure it is locked.

NOTE: To avoid electrical arcing, it is imperative that all surfaces of the buffer and water reservoirs and the plate assembly be dry.

2. Place the assembly onto the plate deck in the correct position (A or B). Firmly push down on the assembly to be sure it is seated completely onto the deck. Close the doors on the 3100 instrument and wait for the instrument to home itself.
3. On the computer, link the plate record with the plate position (A or B) by highlighting the pending plate record and then clicking on the position to be used. Wait while the computer links all records. Click on the green arrow on the menu bar to start the run.

J. Ending a run.

K. When a run is completed, the run folders created can be transferred to the analyst's workstation for analysis and review. If the 3100 instrument is not to be used for longer than one week, the syringes and the capillary array can be removed for storage. (See the ABI PRISM™ 3100 Genetic Analyzer User's Manual for additional storage options.)

L. Removing and cleaning the syringes. (Refer to Chapter 8 in the ABI PRISM™ 3100 Genetic Analyzer Users Manual for additional information regarding cleaning and maintenance.)

1. If the syringe drives are not in the home positions, under "Instrument" from the menu bar, open the "Manual Control" windows and home both the reserve and capillary fill syringes.
2. Unscrew the syringes to remove them from the pump block.
3. Expel any unused polymer into a waste container and dispose of according to the MSDS.
4. Rinse the syringes with warm tap water by drawing water into the syringe and then expelling it several times. Repeat with several rinses of filter-purified water.
5. If desired, the syringes may be dried using compressed air. (This is not required.)
6. Store the syringes with the plunger inside.

M. Cleaning the Pump Block.

1. Within the "Manual Control" window, home both syringes then remove them from the pump block. Under "Manual Control", select "Pin Valve Open" to open the pin valve on the lower polymer block.
2. Remove the previously installed capillary, if the array is to be stored off the instrument.

NOTE: If the capillary has been used for less than 300 injections, it may be stored with both ends in 1X Buffer.

3. Grasp the block with both hands and pull straight out.
4. Rinse the blocks, valve, tubing, and ferrule thoroughly with warm tap water, and then with filter-purified water. A plastic syringe with an adapter may be used for this process.
5. Remove any excess water from inside and outside of the blocks using compressed air and Kimwipes.

6. Verify that the gold electrode socket on the back of the pin valve block is dry and then replace the block. Slide the U-shaped end of the activator arm into the collar at the top of the plunger valve. Make sure the activator arm lines up with the groove in the pin valve before pressing into place. It may be necessary to move the activator arm up or down by selecting "Pin Valve Open" or "Close" from the "Manual Control" menu.

GENESCAN ANALYSIS

All profiles generated from database samples are reviewed by two analysts. The results are compared. Any discrepancies must be resolved prior to CODIS entry.

The ABI 377 DNA Sequencer uses a Macintosh computer platform to collect raw data and also uses Genescan analysis software. A matrix file is applied to the raw data to correct for spectral overlap and produce singular peaks of the four individual colors. A size curve is created using an internal lane standard (ROX 500XL) with DNA fragments of known size. Sample peaks are assigned a size by interpolation.

The ABI 3100 Genetic Analyzer uses a Windows NT computer platform to collect raw data and also uses Genescan analysis software. A matrix created from the spectral calibration of the 3100 is applied, at the point of data collection, to correct for spectral overlap. A size curve is created using an internal size standard (GS-500 LIZ, GS-500ROX) with DNA fragments of known size; sample peaks are assigned a size by interpolation.

A new matrix will be created for each instrument as needed (i.e. if the matrix in use consistently fails to eliminate pull-up or over-subtracts one or more colors). Refer to "Multicomponent Analysis" in the Cofiler, Profiler Plus or Identifiler User Manuals for instructions on how to build a matrix. Once prepared, a matrix will be considered suitable for database analysis if a flat baseline is obtained when it is applied to the matrix standards.

With the ABI 3100 Genetic Analyzer, certain capillaries can fail spectral calibrations and thus, fail to produce matrices. These capillaries can borrow the spectral calibrations of adjacent capillaries that have passed their spectral calibrations. At least twelve capillaries should produce matrices. If not, repeat the spectral calibration process until at least twelve capillaries pass.

NOTE: ***A new matrix must be built following cleaning, maintenance, or replacement of a component of the optics or CCD camera.***

GENESCAN ANALYSIS USING MACINTOSH SOFTWARE

A. Open the Run Folder and locate the Gel File. Open the Gel File and check the tracking to insure accuracy. Retrack if necessary, then re-extract the data and close the file.

B. Open the GeneScan Project.

C. Within the Analysis Control Window, choose the blue, green, yellow, and red colors for analysis. Also insure that the red dye color is marked as the size standard (a diamond symbol should appear in the red boxes).

D. Insure that the analysis parameters are as below:

1. Analysis Range:

   Select a range of data points incorporating both the 75 and 400 bp size standards.

2. Data Processing – Check the following:

   Baseline
   Multicomponent
   Heavy Smooth Option

3. Peak detection:

   B 150
   G 150
   Y 150
   R 150

4. Size Call Range:

   Min 75
   Max 400

5. Size Calling:

   Local Southern Method

6. Split Peak Correction:

   Check none

Note: It may be necessary to adjust the range of data points analyzed in the Analysis Range section of the Analysis Parameters window in order to capture all sizes from 75 bp to 400 bp. In addition, database profiles are derived from single source samples. At the analyst's discretion a Peak Amplitude Threshold of 50 RFUs may be used for low level alleles. The Technical Leader must review and approve any allele calls below 50 RFUs.

E. Highlight to analyze all four colors.

F. Click Analyze.

G. Review the samples individually in Analysis Control.

> ***Note: Once the optimal analysis parameters have been established, the Auto-Analysis function may be used for all runs.***

1. Examine the negative amplification blank and reagent blank for the presence of any contaminating peaks.
2. Examine the positive controls to insure that all peaks are present and sized.
3. Evaluate the samples with the following parameters:
   a. Peak shape and height.
   b. Matrix quality.
   c. Peak profile (examine for artifactual peaks).
   d. Presence of any spurious peaks due to leakage or spillover.

H. Low level samples may be re-analyzed with a lower (down to 50 RFU) peak height threshold. Some samples may require the approval of the Technical Leader.

I. After the review is complete, save any changes.

GENESCAN ANALYSIS USING NT SOFTWARE

A. Begin by copying the run folders of interest onto your workstation's desktop from the shared folder of the server that ties the 3100 to the workstations.

B. Open GeneScan and create a new project by adding sample files of interest from the desktop. Save project as: GSdateanaID(PP/CO)

C. Review a representative number of samples' raw data to determine the Analysis Range.

D. Within the Analysis Control Window, select all colors present for analysis. Insure that the appropriate dye color is marked as the size standard (a diamond symbol should be present in the orange boxes for ID or red boxes for PP/CO).

E. Select the Define New option from the Size Standard pop-up menu next to the sample you wish to use to define the new size standard. Define the standard peaks as: 75, 100, 139, 150, 160, 200, 300, 340, 350, 400, and 450. **Note**: For capillary electrophoresis the 250-peak is labeled as "0".

F. Insure that the Analysis Parameters are as follows:

1. Analysis Range

   Select a range of data points incorporating both the 75 and 400 bp size standards.

2. Data Processing

   Light Smooth Option

3. Peak Detection

   B 150
   G 150
   Y 150
   R 150
   O 150

4. Min Peak Half Width

   2 pts

5. Polynomial Degree

   3

6. Peak Window Size

   19 pts

7. Slope Threshold for Peak start

   0.0

8. Slope Threshold for Peak end

   0.0

9. Size Call Range

   Min 75
   Max 400

10. Size Calling

    Local Southern Method

11. Baseline Window Size

    251 pts

12. Auto Analysis Size Standard

    None selected

G. Highlight all colors present to analyze by clicking on the top left box of the grid.

H. Click Analyze.

I. View results.

   In the Analysis Control window:

   Negative controls: Review the negative controls for the presence of any contaminating peaks.

Ladder: Review the ladder to verify all peaks are called on the electropherogram.

Samples and Positive/Extraction Control: View samples individually and assess overall success of the injection. Examine the following:

peak shape and height

matrix quality

peak profile

size standard – Make sure that the analysis parameter used captures 75-400 bp range, and that the GeneScan size standard peaks are sized correctly.

Check that the Blue, Green, Yellow, and Red peaks for each locus are all present.

In the Results Control window: Verify GS size standard.

Precision: With quick tile off, select size standard color only (can view up to 16 at a time). Align by size and select the "250" basepair peak. Show selected items only and verify that the "250" basepair calls are all within one basepair of each other.

Peak assignment: Verify size standard peaks are assigned the correct types. All peaks assigned a size should end in ".00: (i.e. 75.00 basepair).

Note: It may be necessary to assign a new analysis parameter (AP) and/or size standard for individual samples (or ladders) based on results obtained during analysis (i.e. assignment of new AP to capture 75-400 bp range). In addition, database profiles are derived from single source samples. Therefore the peak amplitude threshold may be lowered to 50 RFUs. The technical leader must review and approve any allele calls below 50 RFUs.

J. After the analysis is complete, save the project.

K. Transfer a copy of the spectral calibration, spatial calibration, run notes, plate record, analysis parameters, and size standards to the run folder.

GENOTYPER ANALYSIS

Genotyper software is used to automatically convert the allele sizes from GeneScan Analysis software into allele designations and to build tables containing the Genotyper information. Genotypes are assigned by comparing the sizes obtained for the unknown sample alleles with the sizes obtained for the alleles in the allelic ladder. The AmpFℓSTR Profiler Plus, Cofiler, Identifiler NT, and Identifiler CODIS NT template files contain the macros that perform the following steps automatically:

A. Find the sample file containing the ladder. (The Sample Info column of the GeneScan sample sheet must contain a unique sample description in order for Genotyper software to build a table. Also, the lanes that contain the allelic ladders must contain the word "ladder" in the Sample Info column of the sample sheet. The Genotyper template identifies the allelic ladder by searching for the word "ladder" in Sample Info.)

B. Create allele size categories that are centered on the sizes obtained for the allelic ladder peaks.

C. Assign the appropriate allele label to sample alleles that fall within the allele size categories.

D. Remove labels from stutter peaks.

E. Build a table containing genotypes for all samples.

Note: If the Sample Info or Color Info field in the sample sheet/plate record was not completed before initiating a run, it is possible to add this information once the data is in the Genotyper application.

1. Under the View menu, choose Show Dye/Lanes Window.
2. Select the first sample by clicking on the row.
3. Select the Sample Info box at the top of the window and type the sample description.
4. Repeat the above steps for every sample in the Dye/Lanes list. Enter the same sample description for each of the dye colors for a single sample.

USING A TEMPLATE FILE

NOTE: The template file is a Stationary Pad, which means that a new document is created each time it is opened. The original template file is not overwritten.

A. Double-click on the template file of interest to simultaneously open the Genotyper application and the template file.

B. Import GeneScan project by selecting Import - from GS File. Select project file from the appropriate Run Folder by double clicking on it. Click finish to import file into Genotyper. Save Genotyper file as: GTdateanaID(PP/CO)

C. From the Macro list at the bottom left of the window, select "Check GS 500". Double-click on this macro, or use command listed, to execute the macro. In the Plot window that appears, scroll through each sample and verify the presence of both the 75 and 400 bp peaks. Confirm correct size calling of the GS internal size standard assigned by GeneScan Analysis software.

D. Execute "Kazam (20% filter)" macro. When finished, a plot window opens containing the labeled alleles for the blue data. The analyst may wish to close this

plot window and sort the data at this point. This can be done by selecting View, Dye lane sorting.

NOTE: The first sample file containing an allelic ladder that is found in the Dye/Lane list is the one that is used by the macro to determine the size of the allele categories that will be used for genotyping. If you use a ladder other than the first ladder, then you must remove the ladder designation from the Dye/Lane list. Simple removal of the "r" will suffice.

E. Examine data by looking through sorted data or by examining by individual color.

EXAMINING DATA

A. Insure that the peaks of the allelic ladder are labeled with the correct allele designations. Peak labeling criteria are defined by the GT template being used. Allele categories and their designations can be viewed in Genotyper under View: Categories. Peaks that do not size within an allele category will have a label indicating "OL Allele" (Off Ladder Allele).

B. Peaks less than the minimum peak height previously specified in GeneScan software should not be labeled. A frequently-encountered GenoTyper anomaly has been the labeling of peaks that are below GeneScan thresholds. These should be manually deleted and noted by the analyst.

C. Scroll through the samples to examine the peak labels and edit the peak labels where necessary. Clicking on a labeled peak will remove the label. Clicking on the same peak again will label the peak by size (bp). Samples that do not meet standards may be removed by opening the dye lanes window, highlighting the sample name, and selecting Clear under the Edit menu.

Note: To change a label, choose Change label from the Analysis menu and select the desired label.

D. Once the samples have been edited, create an allelic table by executing the "Make Allele Table" macro. Keep a print out of the allele table with the sample records. (To print, export the table, then open it in Excel.) Save the project before proceeding (i.e. GTdateanaID(PP/CO).

E. As part of the technical review process, a second reviewing analyst should follow the above steps in the Examining Data section, saving files with his/her initials added at the end (i.e. GT mm/dd/yy anaPP/COrev).

F. Once the typing is confirmed by a technical review, the DNA profiles may be imported into CODIS. See following section on how data is confirmed.

INTERPRETATION OF DATA

INTRODUCTION

The interpretation of database profiles is a matter of both professional judgment and expertise. The following objective criteria are to be used by the analysts to aid in their interpretation of the data presented them. These criteria are based upon validation studies, literature, and experience. They are not a set of hard and fast rules, but rather a means to establish a general framework and outline minimum standards to insure that:

- conclusions are scientifically supported by the analytical data, including that obtained from appropriate standards and controls;
- interpretations are made as objectively as possible; and
- interpretations are consistent from analyst to analyst.

EVALUATION OF STR DATA

The goal of the evaluation and interpretation of the STR data is to correctly determine the DNA profiles from database samples for inclusion into CODIS. The following should be applied to aid in that interpretation.

A peak is defined as a triangular section of the electropherogram that results from the sum of input signals and, as such, shows Gaussian distribution.

The internal size standard (ROX or LIZ) must have the correct sizes assigned to the peaks used for sizing. The 75-350 bp peaks must be captured when using the Cofiler reagent kit. The 400 bp peak must be captured when using the Profiler Plus or Identifiler reagent kits.

The ladder used by Genotyper to call the alleles of the samples analyzed must be correctly labeled with the correct allele designations.

Genotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus.

Homozygous allele peak heights are, in general, approximately twice that of heterozygous peaks as a result of doubling of the signal from two alleles of the same size. Caution should be used with the interpretation of low RFU single allele peaks. They may indicate homozygous alleles, but could also be the result of preferential amplification. Therefore, allelic balance across the sample must be considered in the interpretation process.

Poor amplification at a locus may be due to degraded DNA, presence of inhibitors, extremely low or excessive input DNA or primer mismatch.

Within a single source sample, genotypes generated for D3S1358 and D7S820 in the Profiler Plus and Cofiler amplification and typing systems must be concordant.

Artificial peaks within the analysis range, other than target peaks, may be detected on the electropherograms. These extra peaks may be caused by stutter, incomplete A-addition, pull-up, spillover, or spikes.

STUTTER

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter (n-4) than the corresponding main allele peak. It is also possible, in the case of high target DNA concentration to see n+4 addition and n-8 stutter. These stutter peaks may be due to slippage during amplification. Sequence microvariants can affect the amount of stutter (e.g. a lower amount of stutter is produced from alleles with increased sequence variation between repeats).

Database samples are run with a 20% stutter filter using KAZAM 20% in Genotyper. Any sample giving stutter greater than the 20% should be closely examined and, using the professional judgment of the analyst, determined to be either stutter or an actual peak. The reviewing analyst may help in this call, keeping in mind that it is always possible to rerun or re-amplify the sample to help in this process.

Stutter peaks may be elevated above established thresholds by the following:

- Off-scale raw data (RFU greater than 5000). If the stutter peak is greater than the maximum allowed and the primary peak is above 3000 RFU and/or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be diluted and re-run to resolve the issue.
- For alleles differing by two repeats, the stutter peak from the larger allele may overlap the trailing shoulder of the smaller allele and therefore exhibit an increased stutter percentage. This will not be the case if the smaller allele goes to the baseline before reaching the stutter peak.
- Stutter peaks overlapping an area of elevated baseline may exhibit increased percentages. The analyst may choose to apply a different matrix and re analyze the sample with GeneScan software.

NON-TEMPLATE NUCLEOTIDE ADDITION (A-ADDITION)

AmpliTaq Gold® DNA Polymerase adds an additional nucleotide to the 3' ends of double stranded PCR product. This addition creates a species n+1 nucleotide in length. The n+1 species is considered an allele. In some instances, most notably in situations of sample overload and possibly suboptimal PCR conditions, there can be incomplete addition (n) and two species will result that differ by one base pair (n and n+1). The following considerations should be made to determine if a peak in the n position is an allele:

- Determine if all the alleles are accounted for.
- Determine at which loci the n peaks are present (vWA, D3S1358 and THO1 seem to present this situation most readily).
- Determine if common alleles are known to exist at this locus in the n position.

Samples displaying split peak characteristics (which indicates a high level of incomplete A-addition) may be incubated again at 60ºC for 30-45 min (to complete the A-addition), and/or diluted. The samples should be re-typed.

NOTE: Microvariants may differ by only one base pair so interpretation should be made with caution. Microvariants will not change base pair value upon re-incubation and retyping.

PULL-UP

Small artifactual peaks can appear in other colors under true peaks. This phenomenon is termed "pull-up." Pull-up is the result of spectral overlap between the dyes, which are normally corrected for by the matrix. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at approximately the same size (usually within 0.1 bp but may be more) as the true peak. Pull-up can occur as a result of the following:

- Application of a sub-optimal matrix.
- Amplification using excess input DNA which may lead to off-scale peaks.

SPIKES AND OTHER ANOMALIES

Inconsistencies in the gel or polymer as well as electrical impulses can cause artifactual peaks. Most artifactual peaks can be shown to be false by rerunning the sample. (The peaks fail to show in the same bp size location.) Spikes can occur in one, two, three, or all four (or five) colors. Artifactual peaks occurring in all four (or five) colors are the easiest to diagnose as they will be the same size and of similar height. Artifactual peaks occurring in less than four (or five) colors should be interpreted with caution (especially single color spikes) and the analyst may choose to rerun the sample to insure a clear interpretation. Generally, spikes are thin peaks with variable peak heights, often having the same scan number.

OFF-SCALE DATA

Multicomponent analysis of off-scale data may result in raised baselines, excessive "pull-up" of one or more colors under the off-scale peaks or unnaturally high stutter peak ratios. Analysts may choose to interpret samples with off-scale data (approx. 5000 RFU in unanalyzed data) with the reviewer's approval for CODIS upload. Alternatively, the sample may be diluted and rerun or reamplified and retyped using a smaller amount of target DNA.

OFF-LADDER ALLELES

Peaks not aligning with those in the allelic ladders may be detected both within and outside of the range of the allelic ladders. The Genotyper's software will accurately label many of the alleles not present in the allelic ladders; however, manual genotyping may be used to determine a genotype where Genotyper does not make the assignment. Manual genotypes are assigned using the base pair designations calculated by the GeneScan software. Whenever an off-ladder allele is considered a true off-ladder allele (a variant allele rather than an off-ladder allele due to spikes, pull-up, split peaks, etc.) it

must be confirmed by retyping if it conforms to the same overall guidelines described in the ***Evaluation of STR Data section***.

Off-ladder alleles (OLA) that fall between alleles within the ladder will be designated in accordance with guidelines of the International Society of Forensic Haemogenetics. OLA calls are first converted to sizes in base pairs then compared to the size of the appropriate ladder alleles and the allelic designation determined. If the OLA is not a perfect repeat but rather varies by 1, 2, or 3 base pairs from a ladder allele, then it will be designated as an integer of that variation. For example, if a green OL peak size is 238.89 and the 36 allele of D21S11 ladder is 236.82 bp, then the peak will be designated D21S11 36.2.

Alleles smaller than the lowest molecular weight allele (i.e. A) will be designated <A. Alleles greater than the largest molecular weight allele (i.e. B) will be designated >B. The profile must be confirmed by retyping if it conforms to the same overall guidelines described in the ***Evaluation of STR Data section***. If, upon evaluation of the profile, the allele in question is at a position of an overlap between two loci, then the sample should be analyzed using Profiler Plus and Cofiler.

Off-ladder homozygous alleles are rare and must be interpreted with caution due to the possibility of allelic dropout due to primer binding site mutations. Allelic balance across the profile should be examined. If the peak heights of the off-ladder homozygous allele and the heterozygous alleles are similar, the off-ladder homozygous allele should be considered an uninterpretable locus, and not be entered into CODIS.

PEAK HEIGHT IMBALANCES

Imbalances in peak height at a heterozygous locus may occur. This may be a result of a primer binding site mutation. Samples containing imbalances should be rerun or reamplified to confirm the profile. If the peak height ratio at an unbalanced locus is consistent and is greater than or equal to 35%, the profile may be entered into CODIS as a heterozygote. If the peak height ratio is less than 35%, no alleles for that locus may be entered into CODIS unless approved by the Technical Leader and CODIS Administrator.

TRI-ALLELIC PROFILES

Database samples with three alleles at a single locus may occur. The sample is rerun or reamplified to confirm the type. All three alleles are entered into CODIS. This must be done using the keyboard, since the software packages cannot enter three alleles for a single locus.

Disagreements between analysts regarding interpretation may occur. The analysts may first consult with the Technical Leader. If the allele call(s) remain unresolved, the allele under dispute will not be entered into CODIS.

SPILLOVER AND LEAKAGE (ABI 377)

Database samples are known to be from a single donor. Therefore, in the interest of throughput, database samples may be loaded in adjacent lanes of a gel. During loading of a gel, there is the possibility of a small amount of sample either "spilling over" into an adjacent lane or leaking under the sharkstooth comb into the next lane. Interpretation is possible if the amount of extraneous sample is small. Spillover/leakage must be less than 10% of the adjacent sample peak heights for the profiles to be acceptable. Samples viewed as acceptable by these standards may be rerun at the analyst's discretion.

REAGENT BLANKS

A reagent blank tests for possible presence of contamination of the extraction reagents and/or supplies by an adventitious source of DNA. The adventitious source of DNA may be non-amplified DNA or PCR product. Peaks >50 RFU, not attributable to artifacts, located between 100 and 350 bp or at 75 bp and 400 bp, indicate the presence of contamination and none of the samples extracted or amplified with the reagent blank will be considered for analysis/interpretation. The samples should be reamplified and reanalyzed.

NEGATIVE AMPLIFICATION BLANKS

A negative amplification blank is a test for the possible presence of contamination occurring during the amplification setup. If a negative amplification blank exhibits peaks >50 RFU, not attributable to artifacts located between 100 and 350 bp or at 75 bp and 400 bp, this indicates the presence of contamination and none of the samples extracted or amplified with the negative amplification blank will be considered inclusive for analysis/interpretation. The samples should be reamplified and reanalyzed.

POSITIVE CONTROLS

The positive control should have all the alleles present using a 50 RFU cut-off. They must be genotyped correctly. If alleles are missing or mistyped, the analysis run must be repeated. If this does not solve the problem, the entire set of samples should be reamplified and retyped.

MERGING GENOTYPER ALLELIC TABLE FILES AFTER TECHNICAL REVIEW

The technical review process for database profiles involves merging the typing results obtained by two analysts into one table. The profiles are then compared and any discrepancies in results are resolved prior to CODIS entry.

There are two methods for merging Genotyper allelic files:

A. For the MacIntosh platform, the Genotyper allelic tables from both analysts are imported into Microsoft Excel, merged together and sorted so that corresponding samples are side-by-side. The merged tables are printed, and the profiles from the

two analysts are visually compared to insure that all alleles match. Both analysts must initial the table, indicating that they are in agreement.

B. For the Windows NT platform, CODIS tables are created in Genotyper. In the dye/lanes window remove all controls (RB, NAB, and positive controls), and ladders. Select samples to be removed by highlighting them, then select Clear under Edit. Create a CODIS table by executing the "Make CODIS Table" macro. Export the CODIS table to the file folder, saving the table as: CODISGTdateanaID(PP/CO)TABLE. The reviewing analyst follows the same steps, but includes their initials at the end of the reviewed folder (i.e. CODISGTdateanaID(PP/CO)revTABLE). The two tables are merged together in the NT CMF conversion software, which also compares the results and creates a report listing the file names, specimen names and whether each specimen is accepted or rejected. Any specimen that is rejected must be examined to resolve any discrepancies. The explanation for the rejection and any further actions should be noted on the report, and initialed by both analysts.

CREATING CMF FILES

A CMF file is a common message format file, which contains DNA data in a format suitable for electronic transfer into CODIS. The data includes the specimen name, analyst's name, laboratory ORI, index name, and allele calls for each database profile. The following are the two procedures in use at the CBI for creating CMF files.

CREATING A CMF FILE USING NT SOFTWARE

The Windows NT CMF macro is used to merge the analyst's and reviewer's tables, create a report listing each sample and whether it was accepted or rejected, and convert the information for import into CODIS. Thus it serves two purposes: it is used as a part of the technical review, and then the same file is used to electronically import data into CODIS.

A. Select the CMF convert icon on the desktop or within Excel: The following information is displayed.

File Info: Source Lab- enter your lab ORI
Destination Lab- enter your lab ORI
Read by- Analyst's CODIS user name

Input
Input file 1: Analyst's CODIS table
Input file 2: Reviewer's CODIS table

Output
Save as: dateanaCMF

Error Handling
Select prompt for action for both statements.

B. Select OK to execute merging of specimens.

Specimen details are as follows:
Specimen Category: Convicted Offender
Tissue Type: Blood
Tissue Form: Stain
Population group: Unknown

Click box- "Use these settings for every specimen"

C. Save the report that is generated as: REPORTdateana. Print the report for the review and sample records. Document the rejected samples. Initial any concerns.

D. Transfer the CMF file to a CODIS computer by way of the shared folder on the CODIS server.

CREATING A CMF FILE USING MACINTOSH SOFTWARE

The DataBankSTR software functions solely to convert Excel files into a common message format which can then be transferred into CODIS using the CODIS Import software. Therefore, the technical review of the profiles is accomplished as a separate process.

A. Create a CODIS Genotyper file for the PP and CO runs.

1. Open the appropriate Genotyper file (GT mm/dd/yy/ anaPP) and save it as a CODIS GT file (CODIS GT mm/dd/yy anaPP).
2. Edit the CODIS GT file by removing all ladders and control samples. Leave only the DNA profiles to be imported into CODIS.
3. Run the Make Codis Table macro (command 6). Export the resulting table as CODIS GT mm-dd-yy anaPP to the appropriate Run Folder.
4. Once all CODIS GT tables are created and exported, exit the Genotyper software.

B. Import profiles into DataBankSTR.

1. Launch DataBankSTR and choose NEW from the File menu.
2. Select Insert from the table menu and open the appropriate CODIS GT PP file. The PP profiles for the run will be imported.
3. Select Insert again, and open the corresponding CODIS GT CO file. The CO profiles will be added to the PP profiles.
4. Scroll through the profiles. For each sample check that all alleles are present, and that the results from D3 and D7 are concordant.
5. For those samples with off-ladder alleles, replace "OL allele" with the appropriate allele value.

C. Complete the File Settings.

1. To set the default, go to Default Record under User Information. This should read as follows:

   Sample Info – Untitled
   Sample # - 0
   Category – Convicted Offender
   Tissue Type – Unknown
   Tissue Form – Stain
   Population – Unknown

   NOTE: Default settings are set initially and should not be changed.

2. Choose Untitled Settings from the Table menu and complete as follows:

   User Lab – CBI
   First Name – Analyst Name
   Last Name – Analyst Name
   User Initials – AN
   User ID – Your CODIS user name
   User email – DNACOMM

D. Create a CMF (common message format) file.

1. Choose Export CODIS from the Table menu. This creates the DAT file.
2. Save the DAT file with the run date and analyst's initials (i.e. mmddyyan.DAT). This DAT file will be imported into CODIS.
3. Choose Save As from the File menu to save the DataBankSTR file.

E. Transfer the DAT file to a CODIS computer by way of the shared folder on the CODIS server.

ANALYST ACTIVITY

An analyst activity form is filled out for each sample. Include the following:

LAB/ the CBI case number
SEC/ DBS
ANA/ analyst's initials
DOS/ date of submission
TP1/ DBS
SB1/ DOC, PRO, DYC, or COM
SP1/ 1
TRV/ reviewing analyst's initials

NAMING CONVENTION FOR STR ANALYSIS OF DATABASE SAMPLES

NOTE: The date the samples were run on instrumentation is the basis for naming electronic files.

Date=mm/dd/yy=month/day/year

Sample Number	PCR number + CBI case number Ex. 1000Z005555

MACINTOSH FILE NAMES

Run Folder	Run Folder mm/dd/yy anaPP/CO
Reviewed Run Folder	Run Folder mm/dd/yy anaPP/COrev
Genotyper file	GT mm/dd/yy anaPP/CO
Reviewed file	GT mm/dd/yy anaPP/COrev
CODIS-ready Genotyper file	CODIS GT mm/dd/yy anaPP/CO
Genotyper table	GT mm-dd-yy anaPP/CO
Reviewed table	GT mm-dd-yy anaPP/COrev
CODIS-ready Genotyper table	CODIS GT mm-dd-yy anaPP/CO
DataBankSTR file	mmddyyan
DataBankSTR CMF file	mmddyyan.DAT

WINDOWS NT FILE NAMES

CODIS-ready Genotyper file	CODISGTdateanaID
Reviewed file	CODISGTdateanaIDana2
Genotyper table	GTdateanaIDTABLE
Reviewed table	GTdateanaIDana2TABLE
CMF Conversion Report	REPORTdateana
Excel CMF file	dateanaCMF

CREATING CDs

Each database analyst will back up his/her data for each month on two duplicate CD ROMs. The following should be included for each set of samples, to include all information from both the analyst and the technical reviewer's review of the data:

For the ABI 377 instrumentation

- Run folders
- Genotyper files
- CODIS Genotyper files
- Genotyper tables
- CODIS Genotyper tables
- DataBankSTR files

DataBankSTR CMF files
Sample sheets
Run notes
GeneScan matrix files
GeneScan standard files
GeneScan parameter file

For the ABI 3100 instrumentation

Run folders
GeneScan projects
Genotyper files (contains allele table)
CODIS Genotyper files
CODIS Genotyper tables
CMF conversion reports
CODIS (excel) cmf files
Plate records
Run notes
Spectral and spatial calibrations
GeneScan standard files
GeneScan parameter files

REFERENCES

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CODIS

INTRODUCTION TO CODIS

CODIS stands for COmbined DNA Index System. The purpose of CODIS is to create a national information repository where law enforcement agencies can share DNA information obtained from convicted offenders and forensic evidence. This system allows agencies to cross reference case evidence profiles with those of other agencies and to generate investigative leads by comparing forensic DNA profiles with profiles from convicted offenders. All criminal justice agencies are eligible to participate in CODIS if they perform DNA analysis using specific loci and adhere to the NDIS Standards for Acceptance of DNA Data. CODIS accepts DNA profiles derived from either RFLP analysis or PCR analysis.

Currently, there are three levels of CODIS: Local DNA Index System (LDIS), State DNA Index System (SDIS), and National DNA Index System (NDIS). Each local system contains databases consisting of DNA profiles analyzed at that laboratory. The local systems all upload the DNA profiles from their databases to the designated state system (each state has one designated SDIS location). Thus, the state index consists of profiles collected from all participating LDIS laboratories. The State Administrator uploads all appropriate profiles contained in SDIS to the national system. The Federal Bureau of Investigation (FBI) maintains the national system.

CBI has three local systems, located in the Denver, Pueblo, and Montrose laboratories. CBI Denver laboratory is also designated as the SDIS location for Colorado. CBI maintains two CODIS databases (or indexes): the Convicted Offender Database and the Forensic Unknown Database. (The Unidentified Human Remains Database and the Relatives of Missing Person Database are not currently in use at CBI.) Profiles are entered by the local laboratory into the appropriate LDIS database. The local systems upload profiles to SDIS in the CBI Denver lab. The state level is then responsible for uploading the collected profiles to the national level.

At CBI, each of the three laboratories analyzes and enters DNA profiles from both forensic evidence and convicted offenders. Forensic unknown DNA profiles may be searched against other forensic unknown profiles and convicted offender profiles in that local system. These searches may result in local case-to-case links or generate investigative leads in the event of a hit against the Convicted Offender Database. Upon upload to the state system, those DNA profiles will be searched against forensic unknown profiles from other local laboratories as well as all persons contained in the statewide Convicted Offender Database. Profiles from convicted offenders containing all 13 CODIS core loci and profiles from forensic unknown samples with a minimum of 10 of the CODIS core loci are acceptable for upload into NDIS. These profiles will be compared to the forensic unknown profiles and convicted offender profiles from other states contained within NDIS.

Forensic DNA laboratories established by local law enforcement agencies may participate in CODIS as an LDIS location. Each laboratory must adhere to the NDIS Standards for Acceptance of DNA Data to become eligible. They must apply through the State Administrator to the FBI. If approved, the FBI will install CODIS and that laboratory may upload forensic profiles to SDIS.

ORGANIZATION AND MANAGEMENT

FORENSIC DNA ANALYST

A. The forensic DNA analyst generates DNA profiles from forensic cases and determines whether or not a DNA profile obtained from a particular case will be entered into LDIS.

B. The analyst communicates his/her determination through a copy of the final report and an appropriately marked and technically reviewed STR summary sheet.

C. Each forensic analyst must enter the appropriate profiles from his/her case into CODIS. The audit trail created during data entry must indicate that the person entering the profile(s) is the person who performed the analysis.

D. It is the responsibility of the analyst to notify the CODIS Administrator of any CODIS related actions on their casework.

DATABASE DNA ANALYST

A. The database DNA analyst generates DNA profiles from the convicted offender cases and, upon the completion of a technical review, enters those profiles into LDIS.

B. It is the responsibility of the analyst to notify the CODIS Administrator of any CODIS related actions on their Convicted Offender Database samples.

LOCAL (LDIS) CODIS ADMINISTRATOR

A. The Local CODIS Administrator, along with the Technical Leader, is responsible for ensuring that the DNA section is in compliance with all aspects of the NDIS Standards for Acceptance of DNA Data, including quality assurance of the data.

B. The duty requirements for the Local CODIS Administrator are as follows:

1. The Local CODIS Administrator must possess a Bachelor's degree in a natural science or computer science.

2. The Local CODIS Administrator shall have a working knowledge of computers, computer networks, and computer database management.

3. The Local CODIS Administrator shall have an understanding of DNA profile interpretation.

4. The Local CODIS Administrator functions as the system administrator of the laboratory's CODIS network and is responsible for all operations of the local CODIS system. This responsibility includes but is not limited to:
    a. software updates,
    b. security of the DNA profile data stored in CODIS,
    c. training analysts in the use of the CODIS system,
    d. tape backups of the system,
    e. uploading DNA profiles into SDIS,
    f. processing outside agency search requests,
    g. maintaining monthly hit records and forwarding them to the State CODIS Administrator,
    h. maintaining records as required by the State CODIS Administrator,
    i. ensuring that submitting agencies are notified of hits regarding their cases
    j. ensuring that both the State Administrator and CBI Deputy Director are informed of hits within their laboratories.

C. The Local CODIS Administrator has the authority to terminate the laboratory's participation in CODIS in the event of a problem until the reliability of the computer data can be assured.

STATE (SDIS) CODIS ADMINISTRATOR

A. The State CODIS Administrator, along with the Technical Leader, is responsible for ensuring that the DNA sections in all participating local CODIS laboratories are in compliance with all aspects of the NDIS Standards for Acceptance of DNA Data, including quality assurance of the data.

B. The State CODIS Administrator must satisfy all duty requirements and responsibilities of the Local CODIS Administrator, and functions as the Local CODIS Administrator for the CBI Denver laboratory.

C. Additional responsibilities of the State CODIS Administrator include but are not limited to:
    1. processing the LDIS to SDIS uploads from each local laboratory
    2. routine autosearches
    3. processing remote search requests
    4. sending the SDIS to NDIS upload weekly
    5. processing outside agency search requests

6. obtaining the local laboratories' hit records, maintaining hit records for the CBI Denver local laboratory, and forwarding hit counts for the State of Colorado to NDIS on a monthly basis
7. maintaining user information and informing the FBI of any changes
8. responding to any and all requests for information from the FBI
9. completing paperwork for putting new laboratories on the system
10. keeping abreast of any software or hardware recommendations
11. training of new analysts and Local CODIS Administrators
12. notifying the CBI Deputy Director of NDIS hits

D. The State CODIS Administrator has jurisdiction over all CODIS laboratories in the State of Colorado, and has the authority to terminate any laboratory's participation in CODIS in the event of a problem until the reliability of the computer data can be assured.

DNA PROFILE MANAGEMENT

APPROVED DNA PROFILES FOR LDIS

Samples to be entered into LDIS must have been obtained while following all of the NDIS Standards for Acceptance of DNA Data. These DNA profiles must be obtained using a validated standard operating procedure that utilizes an NDIS accepted PCR kit. The positive control must have been typed correctly and there must be no signs of contamination.

For forensic profiles, only the alleles that are attributed to the putative perpetrator(s) may be entered into LDIS. Alleles derived from forensic profiles that are unambiguously attributed to a victim or individuals other than the perpetrator(s), such as, but not limited to a husband or boyfriend, SHALL NOT be entered into LDIS.

DNA profiles from convicted offenders must be obtained from standards collected in accordance with current Colorado statutes.

The following standards shall be used to determine if a profile qualifies for LDIS/SDIS database entry:

A. Forensic unknown samples recovered from evidence in a case where there is no identified suspect (non-suspect cases).

B. Forensic unknown samples, which have been compared to a suspect identified in the case, regardless of whether the suspect is included or excluded.

C. Profiles obtained from convicted offenders meeting current statutory requirements for inclusion in the Convicted Offender Database.

D. Forensic unknown mixtures

1. If a mixture occurs that can be easily separated as known and unknown contributors, only the alleles consistent with the unknown individual(s) will be entered into LDIS. (For example, a vaginal swab that has a 4 allele pattern in which 2 of the alleles are consistent with the victim in the case. The alleles consistent with the victim may be removed from the mixture leaving the remaining alleles to be entered into LDIS.)
2. Once the alleles from the known contributor are removed from the profile, resulting single source DNA profiles may be entered into the Forensic Unknown Database.
3. For more complicated mixtures, care must be taken to determine which alleles (if any) should be entered into CODIS. There may be instances in which all alleles or none of the alleles in the mixture profile will be entered into LDIS.

E. Elimination standards or forensic unknowns matching elimination standards will not be entered into CODIS.

F. Victim standards or forensic unknowns matching victims will not be entered into CODIS.

G. Suspect standards will not be entered into CODIS.

H. DNA analysts will use their discretion in regard to any other DNA profiles that might be generated during the course of their analysis that do not fit into any of the above categories, e.g., a DNA profile from a bloodstain that may or may not have anything to do with the crime.

I. Off-ladder alleles that vary by less than the consensus repeat unit will be designated as an integer of that variation (.1, .2, .3, or .X may be used) for entry into CODIS. (Example: TH01 8.2 allele.)

J. If an allele falls above the largest or below the smallest peak of the ladder of the NDIS acceptable alleles, the allele will be designated as either greater than (>) or less than (<) the respective ladder allele for entry into CODIS.

K. For NDIS acceptance, profiles derived from forensic unknown samples must have a minimum of 10 of the CODIS core STR loci. Profiles from convicted offender samples must have all 13 CODIS core loci.

SAMPLE INPUT PROTOCOL - CASEWORK

A. A forensic case may have more than one sample with the same DNA profile. A single profile will be entered for each different DNA profile obtained in the case. The first exhibit number profile, or the most complete profile from the analysis, will be entered. (i.e. if four bloodstains on a pair of jeans, X1-3-1 through X1-3-4, yield the same DNA profile, only X1-3-1 will be entered into LDIS).

B. The following format for Specimen ID# will be used for all CBI cases:

1. Forensic Unknowns: **Exhibit#Case#** (i.e. 1D000000) There are to be no spaces or punctuation.

C. Profiles from cases will be entered using the following procedure.

1. Log on to CODIS Local by entering you CODIS User ID and password. (Do not enter profiles under another analyst's name.)
2. Under PCR Analysis, click on the PCR button. This opens the Select/Add PCR Specimen screen.
3. Enter the Specimen ID# in the Specimen ID blank. Check the entry for accuracy.
4. Select the applicable Specimen Category (e.g. Forensic, Unknown) by scrolling through drop-down menu, then highlighting the selection. This specifies the Index for that profile.
5. Check that the analyst's name next to Assigned To is correct. If not, repeat the Log on process using the name of the analyst performing the profile entry.
6. Click on the Add button.
7. Enter allele calls into the table under Reading Information. Click on the blank box under each locus and enter the alleles for that locus, separating the individual alleles with a comma. Do not use spaces. The first and second reading for each locus must match.
8. Mark the profile for transfer to LDIS and SDIS by clicking on LDIS and SDIS in the Reading Information table. A green check mark must be in the box for each locus, indicating that the alleles are marked for transfer.
9. Click on the Transfer to LDIS button.

   The profiles are now in LDIS.
10. Click on the Clear button to clear the entry screen for additional profiles.
11. Exit all screens when profile entry is complete.

D. Upon completion of a forensic case, the analyst will determine which sample(s) and which alleles in each sample will be entered into LDAS. Profiles must be technically reviewed prior to transfer from LDAS to LDIS.

E. Each analyst will be responsible for his/her case profile entry into the appropriate database in CODIS.

F. Binders containing copies of DNA case reports will be maintained at each laboratory. Once the sample has been entered into CODIS, the copy of the report and the STR allele worksheet will be filed into the DNA case binders.

NOTE: Copies of the case reports from all cases, including those not entered into CODIS, must be filed in the DNA case binders.

G. When appropriate, the case report will reflect that a search was performed and that the sample will be searched routinely.

H. Only DNA Analysts who have passed the FBI background check will enter profiles into CODIS.

SAMPLE INPUT PROTOCOL – DATABASE

A. Technically reviewed database profiles may be imported into CODIS as CMF (common message format) files and transferred into LDIS using the batch transfer function. Each analyst is responsible for transferring his/her own data into LDIS. Alternatively, individual profiles may be entered using the keyboard entry procedure described for casework profiles (the Convicted Offender Specimen Category should be selected).

B. The following Specimen ID # format shall be used for database samples: **PCR#Case#** (i.e. 1000Z000000). There are to be no spaces or punctuation.

C. Only DNA Analysts having passed the FBI background check will enter profiles into CODIS.

D. The following is the procedure for importing CMF files into LDAS:

1. Log on to CODIS Import by entering your CODIS User ID and Password.
2. Select a file to import.
    a. Click on the file open icon.
    b. Select the proper CMF file.
    c. Click on OK.
3. Validate the selected file.
    a. Click on the validate icon. CODIS will perform the validation.
4. Examine the Import Status results.
    a. Click on the printer icon.
    b. Choose screen.
    c. The action taken for a particular profile will be described. A red message indicates problems with the data
    d. Close the Import Status results file.
5. Import the CMF file.
    a. Click on the import (arrow) icon. CODIS will import the profiles.

6. Examine the Import Status results.
    a. Click on the printer icon.
    b. Choose screen.
    c. The action taken for a particular profile will be described. A red message indicates problems with the data.
7. Close the Import Status results file.
8. Exit from CODIS Import.

E. The profiles are now in LDAS.

F. Transferring Profiles from LDAS to LDIS
1. Log on to CODIS Local by entering the User ID and Password.
2. Choose PCR Analysis by clicking on PCR.
3. Click on Batch Transfer.
4. Bring up imported profiles for transfer by choosing the following:
    a. Specimen Category – Convicted Offenders
    b. Assigned To – Analyst's Name
    c. LDIS Filter
    None in LDIS – for new specimens
Some in LDIS – for old specimens
5. Click Query. A blue background should highlight all profiles.
6. Check that all loci are marked with a white X.

G. Click on Start Transfer. All blue highlighted profiles will be transferred to LDIS.

H. Close Batch Transfer.

I. Exit from CODIS Local.

J. The profiles are now in LDIS.

CODIS SEARCHES AND UPLOADS

A. If necessary, a search of the database will be conducted before a DNA case is reported. The DNA analyst who processed the case is responsible for initiating the search. The results of this search should be reported to the local agency by way of the case report. There are two mechanisms available for searching.
1. Analysts may use Searcher under the State programs to search their Local GDIS database.

2. Analysts may request a remote search of the SDIS database. The profile is entered into LDAS (it is **not** marked for transfer to LDIS). The remote search request is then sent to SDIS and executed by the State Administrator.

B. In general, ALL samples once entered into LDIS will be marked for SDIS.

C. An SDIS upload from each local laboratory may occur weekly in conjunction with the State system upload to NDIS. The Local CODIS Administrator must conduct the SDIS upload.

D. Routine SDIS autosearches will be performed.

COMMUNICATION PROTOCOL FOR A POSSIBLE CODIS HIT

NOTE: The AIC and the Deputy Director of CBI will be notified of CODIS hits resulting in investigations aided.

NOTE: Release of personal information by the CBI is in accordance with the following federal legislation: The DNA Identification Act of 1994 and the Privacy Act. Additionally, C.R.S. 24-72-305, which governs public inspection of criminal justice records, denies public access to the results of DNA testing pursuant to state statutes regarding Convicted Offender samples.

A. Upon conducting a search of the LDIS index, a possible hit (match) is made. The following course of action shall be taken:

1. A copy of the match report will be printed. This report will give the case designation for the case to which the possible match was made.

2. The case file(s) for the cases involved will be obtained. The analysts involved with the cases will be notified. The analyst(s) who processed the case will review the DNA profile(s) entered into CODIS for confirmation.

3. If the hit involves a convicted offender profile, the sample from that individual will be re-extracted and re-analyzed to confirm the match.

4. Investigating officers will be notified. The case reports will reflect that the officers were notified of the hit. A copy of the CODIS match report will be placed into each of the case files.

5. The hit will be recorded on the CODIS hits worksheet as well as the hit scorecard.

6. The State CODIS Administrator in Denver will be notified by e-mail of any hits made at the Local level. The hit will be added to the State hit total. Copies of the following will be forwarded to the State CODIS Administrator: the CODIS match report, the case report, the STR allele worksheet, the case submittal sheet, and any written correspondence regarding the hit.

B. If a possible hit occurs at the State level (SDIS):

1. The State Administrator will inform the Local Administrator. A copy of the hit report will be forwarded, or a match message will be sent.
2. The DNA analyst(s) involved will be notified, so that the DNA profiles can be reviewed.
3. If the match involves a convicted offender profile, re-testing will be performed.
4. Investigating officers will be notified. Reports will be issued to reflect the notification of the officers of the hit. A copy of the CODIS match report will be placed into each of the case files.
5. Copies of the case report, STR allele worksheet, the case submittal sheet, and any written correspondence regarding the hit will be sent to the State Administrator.
6. The hit will be recorded on the CODIS hits worksheet as well as the hit scorecard.

C. If a possible hit is made as a result of an outside agency search request (fax request or keyboard search):

1. The analyst involved in the case will be notified, so that the DNA profiles can be reviewed.
2. The outside agency will be notified of the potential match by telephone or letter.
3. The results will be discussed. The CBI analyst will determine, along with the outside agency analyst, if further DNA analysis is required. The analyst's course of action will be documented in the case file.
4. If the hit results in an investigative lead, the identifying information for the DNA profile will by forwarded to the outside agency.
5. It is the responsibility of the agency requesting the search to notify the investigating officers.
6. The CODIS Administrator will keep copies of the match report and any other documentation. The hit will be classified using the CODIS hit counting guidelines. The NDIS Custodian will be notified of any interstate hits in writing per NDIS procedures.

D. If a possible hit occurs at the National level (NDIS interstate hits):

1. The analyst involved in the case will be notified, so that the DNA profiles can be reviewed.

2. The NDIS Procedures Manual lists specific instructions for contacting CODIS laboratories, issuing reports, and notifying the NDIS Custodian in the event of an interstate hit. The CBI will follow NDIS procedures in the event of an NDIS hit.
3. It is the responsibility of the CODIS laboratory that submitted the matching profile to notify the investigating officers of the hit and to provide them with contact information for the appropriate out-of-state agency.
4. The hit will be classified using the CODIS hit counting guidelines.

In the event of a hit against a profile in the Convicted Offender Database, the identification information for that sample may be verbally released to the appropriate agency or investigator(s), prior to re-analysis of the sample. The investigating officer(s) or agency should be informed that the hit is not confirmed, and that the laboratory will contact them once the confirmation process has been completed.

The identification information for the Convicted Offender Database is contained in a file on a stand-alone computer in the Denver laboratory. In the event of a hit against the Convicted Offender Database, the identification information for the profile is released through the Denver laboratory.

DELETING CODIS PROFILES

A. It may be necessary to delete a record from LDAS or LDIS. A hard copy of the LDAS and LDIS delete reports will be printed. The analyst who performed the deletion will initial the reports and forward the record to the CODIS Administrator for that laboratory.

B. Samples found to have been sent to the CBI in error may be removed from CODIS at the discretion of the State Administrator. The State Administrator will maintain documentation supporting the removal of a sample.

REQUESTS FOR EXPUNGEMENT

The CBI expungement procedure for the removal of DNA profiles from CODIS follows the requirements of the DNA Analysis Backlog Elimination Act of 2000. Section 6 (d) EXPUNGEMENT OF RECORDS of the Act provides, in pertinent part:

"(2) BY STATES - (A) As a condition of access to the index described in subsection (a) [referring to the national DNA index], a State shall promptly expunge from that index the DNA analysis of a person included in the index by that State if the responsible agency or official of that State receives, for each conviction of the person of an offense on the basis of which that analysis was or could have been included in the index, a certified copy of a final court order establishing that such conviction has been overturned.

(B) For purposes of subparagraph (A), a court order is not 'final' if time remains for an appeal or application for discretionary review with respect to the order."

Therefore CBI requires both a written request and a certified copy of a final court order as defined above to implement the expungement procedure.

The written request and the court order directing the expungement must be issued to CBI. It then must be reviewed and verified by the AIC of the DNA section to which the order was directed, prior to proceeding with expungement. Upon this approval, the CODIS Administrator of the laboratory that entered the record shall be responsible for:

A. Deleting all DNA profiles, records and identifiable information in the CODIS database pertaining to the person with regard to the dismissed or reversed conviction.

B. Destroying all samples obtained from the individual that pertain to the dismissed or reversed conviction.

C. Deleting the profile and sample name from the appropriate DNA analysis files.

D. For convicted offenders, deleting the name, date of birth, sex, and DOC/ML/DYC identifiers from the Offender Inventory Log. Replace the name with "NO RECORD – EXPUNGED." Place the date of expungement under date of birth. DO NOT RELEASE ANY INFORMATION FOR THE SAMPLE. A "NO RECORD" RESPONSE MUST BE GIVEN.

E. Placing a copy of the expungement order in the case file.

F. Notifying the State CODIS Administrator and provide a copy for the state records.

The State CODIS Administrator shall notify the NDIS Custodian of the expungement and forward the appropriate paperwork.

NDIS QUALITY ASSURANCE/QUALITY CONTROL STANDARDS

PROFICIENCY TESTING

A. All qualified DNA technicians and analysts conducting casework will participate in two external proficiency tests per year. These tests will be conducted on a semiannual basis at regular intervals not to exceed 183 days, as per the FBI Quality Assurance Standards.

B. Any questions/problems related to a proficiency test will be handled as detailed in the *CBI Forensic Laboratory Quality Manual*.

C. All QA/QC guidelines outlined in the CBI Forensic Laboratory DNA Analysis SOP and the CBI Forensic Laboratory Quality Manual will be followed.

D. Proficiency test documentation will be provided as specified by current NDIS procedures.

AUDITS

A. The DNA sections will be audited once a year. There shall be not less than six months or more than eighteen months between audits. External audits will be performed during alternate years.

B. Audit documentation will be provided as specified by current NDIS procedures.

SYSTEMS OPERATIONS

CODIS SECURITY

The CODIS servers are located in locked rooms with limited access. Individual workstations are located in analysts' offices. The offices are within the CBI laboratories, behind a keypad door in a secure building. The following security procedures have been implemented:

A. The CODIS workstation must be logged on and off each time CODIS is used.

B. Only FBI-approved personnel have User Id names and passwords for CODIS. Persons other than those approved by the FBI are not authorized to have access to the CODIS database.

C. The CJIS/WAN router for CBI Denver laboratory (the SDIS router) is located in the locked central computer room.

D. ALL CODIS users are responsible for the security of the software.

TAPE BACKUP PROCEDURES

The CODIS servers are set up to do a nightly tape backup. Both the Local CODIS Administrators and the State CODIS Administrator will do two full tape backups once a month. One will be stored in each laboratory. The second will be stored off site.

FILE STORAGE

A. Copies of DNA case reports and the associated tables will be stored in the DNA laboratory. The DNA laboratory is secured behind a keypad door within a secure building.

B. The file containing the identification information for the Convicted Offender Database is kept on a stand-alone computer at the Denver laboratory. Access to this computer is limited to the persons involved with the Convicted Offender Database. Backups of the personal information are performed whenever entries are made. Contents of this file will be compared to the specimen numbers entered into CODIS on a yearly basis, for quality assurance purposes.

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DNA DATABASE

Database (DBS) samples may consist of whole blood, buccal swabs, or prepared cards required for genetic testing mandated by legislation. DBS submissions are received from the Department of Corrections, Probation, Division of Youth Corrections and Community Corrections for entry into the DNA database only. No report is generated and submitted samples are not returned.

SUBMISSION, PREPARATION, AND STORAGE OF SAMPLES FOR OFFENDER DATABASE

A. Submission Procedures

1. Samples

   a. Blood samples must consist of at least one 4ml Lavender (purple top EDTA) tube from each offender.

   b. Buccal samples must be dried on the appropriate substrate and then sealed in a protective package.

2. Submission, Labeling And Packaging

   a. The blood tube is labeled with the offender's name (first & last). The submitting agency case number, date the blood was drawn, and the initials of the person collecting the sample are additional identifiers that are preferred, but not required. If no identifiers are present on the blood tube, the tube is not analyzed and a redraw request is made.

   b. The protective packaging containing the buccal sample is labeled with the offender's name (first & last). The submitting agency case number, the date the sample was collected, and the initials of the person collecting the sample are additional identifiers that are preferred, but not required. If no identifiers are present on the saliva sample, the sample is not analyzed and a request for another sample is made.

   c. The CBI Database Submission Form must accompany a DBS sample. The form will be kept separate from the blood tube or buccal sample and its sealed container.

   d. If a blood tube is broken or leaking at time of submission to CBI, the sample will not be accepted or will be destroyed.

   e. If the sample does not meet the above requirements, CBI personnel will use discretion as to whether the sample will be analyzed.

   f. At the time of sample submission to the CBI Denver Laboratory, a signature is required. CBI Personnel (an evidence technicians, an

AIC, or a criminal investigator assigned to the database section) will stamp the chain of custody with the date received and assign a case number to each sample. Once the samples have been assigned a case number, they are labeled and stored.

g. Samples requiring refrigeration will be stored in a refrigerator located in the CBI Serology section.

CBI HANDLING OF DATABASE SAMPLE SUBMISSIONS

A. Data Entry

1. The CBI Database Submission Forms are checked for errors and omissions. Name, date of birth, agency case number, offense code and signature are required. If any required information is not present, the submitting agency is notified and the information needed is obtained. All data is entered into the stand-alone DNA database computer. Every time data is entered into the computer, a back-up disk is created.

2. Periodically a hard copy of recent submissions is generated and used to record which samples have been analyzed, by whom, and which remaining samples require testing. This printout is stored in the STR Binder located in the Denver DNA prep area.

B. Preparation Of Stain Card From Liquid Blood

1. A stain card is prepared for each blood sample. Each stain card contains the offender's name, agency case number, case number, and a unique confidential PCR number assigned by CBI personnel.

2. Preparation of FTA™ blood stain cards is carried out in a manner to minimize risk of contamination. The precautions include:

   a. All blood stain cards are prepared in a hood. The individual preparing the card should wear protective eyewear and clothing.

   b. The FTA™ cards are handled wearing gloves. Gloves are changed when contact with blood may have occurred.

   c. Each new stain card is prepared on a clean surface.

   d. A fresh lab tissue is used to open each EDTA blood tube.

   e. Each FTA™ card is spotted with a fresh sterile transfer pipet. One to two drops of blood is placed within the circle of the card.

   f. Prepared stain cards must not come in direct contact with each other or with unprepared cards.

3. The blood spotted stain card is left in the covered hood to dry (at least one hour).

4. Upon completion of preparing the stain card, the offender's blood tube and any remaining blood are disposed of following the procedures established by the biohazardous waste disposal contractor for the site.

5. Once the spotted stain card is thoroughly dry, the card is packaged in a plastic sleeve, heat-sealed with the preparer's initials & date and stored appropriately. The FTA™ cards are stored in a locked cabinet at room temperature. The S & S™ cards are stored in a locked -70°C freezer.

C. INTERLAB TRANSFER OF SAMPLES

1. DBS samples may be transferred between CBI laboratories to facilitate the effective use of personnel, resources and materials. DBS samples are transferred by common carrier (e.g.: UPS or Federal Express) or by an agent of the Bureau traveling to a particular site.

2. Whole blood must be kept refrigerated. Prepared stain cards made on FTA paper may be kept at room temperature.

3. Each DBS sample must be transferred with the corresponding working copy from the CBI Database Submission Form. There is no internal chain of custody and no signature required for interlab transfers or upon completion of analysis.

4. The laboratory assigned to analyze the sample will store the blood stain card or buccal sample upon completion of analysis.

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APPENDIX

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APPENDIX A

ANALYST ACTIVITY (AA) FORMS

AA Codes

HOMicide

TP1/:	**HOM**	
SB1/:	NCD	Included suspect
	XCD	Excluded suspect
	ROU	Routine – sample run, no inclusion or exclusion
	NSF	Samples Not Sufficient for analysis
EX1/:		Number of Examinations
SP1/:		Number of Specimens
HR1/:		Hours per SOP

SEXual Assaults

TP1/:	**SEX**	
SB1/:	NCD	Included suspect
	XCD	Excluded suspect
	ROU	Routine – sample run, no inclusion or exclusion
	NSF	Samples Not Sufficient for analysis
EX1/:		Number of Examinations
SP1/:		Number of Specimens
HR1/:		Hours per SOP

OTHer Crimes

TP1/:	**OTH**	
SB1/:	NCD	Included suspect
	XCD	Excluded suspect
	ROU	Routine – sample run, no inclusion or exclusion
	NSF	Samples Not Sufficient for analysis
EX1/:		Number of Examinations
SP1/:		Number of Specimens
HR1/:		Hours per SOP

DataBaSe

TP1/:	**DBS**	
SB1/:	DOC	Dept. of Corrections
	PRO	Probation
	DYC	Division of Youth Corrections
EX1/:		Number of Examinations
SP1/:		Number of Specimens

TRV – Technical ReView

TP1/: **TRV**

AA Forms: Counting Exams

DNA: Blood, Hair, or Tissue Specimen/item

STR = 10 exam/item

PMDQα = 5 exam/item

DNA: Differential Extraction Specimen/item

STR = 20 exam/item

PMDQα = 5 exam/item

APPENDIX B

TERMS AND DEFINITIONS FOR CODIS

A. **Autosearcher** – A CODIS program which automatically searches all DNA profiles in a user-specified index against all profiles in one or more other user specified indexes.

B. **Candidate Match** – A possible match between two or more DNA profiles discovered by CODIS software. Qualified DNA analysts must verify candidate matches.

C. **CODIS** – COmbined DNA Index System – The entire system of DNA indexes maintained at the national, state, and local levels.

D. **CODIS Administrator (Local or LDIS)** – Responsible for overseeing the CODIS system. Duties may include: organization of CODIS materials, installing software, routine tape backup, system QA, routine local searches, and overseeing verification of matches involving profiles entered into their system. The Local CODIS Administrator is also responsible for SDIS uploading.

E. **CODIS Administrator (State or SDIS)** – Responsible for overseeing the CODIS system. Duties may include: organization of CODIS materials, installing software, routine tape backup, system QA, routine local searches, statewide autosearches, and training of Local Administrators. The State CODIS Administrator is also responsible for NDIS uploading, verification of matches, and certifying that all CODIS laboratories in the state are in compliance with NDIS requirements.

F. **CODIS Import** – Import is a Windows program that copies DNA data from a Common Message Format (CMF) data file and places the data into LDAS.

G. **CODIS core loci** –The following STR loci constitute the 13 core loci that are required for a complete PCR profile: CSF1PO, FGA, THO1, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11.

H. **CODIS User** – A CODIS user is anyone who has access to CODIS and has passed the FBI background check. The State Administrator adds users to NDIS.

I. **Forensic Unknown sample** – A biological sample that is found at the scene of a crime that does not appear to have originated from the victim or any elimination individuals.

J. **GDIS** – Generalized DNA Index System. The software and database used for LDIS, SDIS and NDIS.

K. **Hit** – A confirmed match between two or more DNA profiles discovered by CODIS software at a single instant in time. Hits may occur at any level in the CODIS hierarchy, LDIS, SDIS, or NDIS. There are two categories of hits:

   A **Forensic Hit** (FH) occurs when two or more forensic samples are linked at LDIS, SDIS, or NDIS. Forensic hits are sometimes called case-to-case hits.

An **Offender Hit** (OH) occurs when one or more forensic samples are linked to a convicted offender sample at SDIS. Offender hits are sometimes called case-to-offender hits.

L. **Investigation Aided –** This occurs when a CODIS hit provides information that assists a criminal investigation.

M. **Keyboard Search –** A manual search of CODIS initiated by a CODIS user.

N. **LDAS –** Local DNA Analysis System - The data entry part of CODIS. Profiles and the accompanying information can be entered into LDAS either by using the computer keyboard for hand entry or using CODIS Import for electronic transfer.

O. **LDIS –** Local DNA Index System - The locally administered, centralized system of DNA identification records containing forensic unknowns and convicted offender profiles.

P. **Match Report** – After CODIS determines that two or more DNA profiles match, this electronic report is generated by CODIS and automatically distributed to the laboratories responsible for matching profiles.

Q. **Match stringency** – Determines whether or not two DNA profiles match. CODIS supports three levels of match stringency: low, moderate, and high.

Low – Matches occur when one or more allele match between the target and candidate profiles at a given locus. All alleles do not have to match at low stringency.

Moderate - Matches require all alleles to match, but the target and candidate profiles can contain a different number of alleles. That is, if the target profile has two, then two alleles must match. If the target profile has just one allele then a candidate may have two alleles. This match stringency is used to search the Forensic Mixture Database.

High – All alleles must match between the target and candidate profiles. This match stringency is used to search the Forensic Unknown Database.

R. **NDIS** – National DNA Index System – The FBI administered, centralized system of DNA records contributed by all State and Local participating laboratories. NDIS receives records from every lower level index and supports the searching functions at a National level.

S. **NDIS Audit Review Board** – A board, convened by the FBI, that has the responsibility of reviewing external audit results from NDIS participating laboratories.

T. **Non-Suspect Case** – A case is considered a non-suspect case when it falls into one of the following categories:

1. A case without a suspect listed, that is analyzed for DNA.
2. A case in which DNA analysis has been conducted and the listed suspect is excluded.

U. **SDIS** – State DNA Index System – The centralized system of DNA identification records contributed to the state by all local participating laboratories.

V. **Suspect** – An individual whose identity is known to the police and who is suspected to be the perpetrator of a crime.

W. **Uploading –** The movement of DNA profiles between systems at different levels.

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APPENDIX C

RESOURCES

GENERAL RESOURCES

The procedures described here are derived from a variety of sources. Portions of the protocol come directly from some of the references cited below.

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APPENDIX D

ALLELE FREQUENCIES[2]

D3S1358

Allele	African American (N = 210)	Caucasian (N = 196)	Southwest Hispanic (N = 191)	Southeast Hispanic (N = 191)
<12	0.0119	0.0123	0.0120	0.0131
12	0.0119	0.0123	0.0120	0.0131
13	0.0119	0.0123	0.0120	0.0131
14	0.1214	0.1404	0.0790	0.0838
15	0.2905	0.2463	0.4258	0.3534
15.2	0.0119	0.0123	0.0120	0.0131
16	0.3071	0.2315	0.2656	0.2461
17	0.2000	0.2118	0.1268	0.1623
18	0.0548	0.1626	0.0837	0.1387
19	0.0119	0.0123	0.0144	0.0131
>19	0.0119	0.0123	0.0120	0.0131

[2] These frequency tables are from the *Short Tandem Repeat Analysis Protocol* (Federal Bureau of Investigation Laboratory, 7/00)

ALLELE FREQUENCIES (CONTINUED)

vWA

Allele	African American (N=180)	Caucasian (N=196)	Southwest Hispanic (N=203)	Southeast Hispanic (N=240)
<11	0.0139	0.0128	0.0123	0.0104
11	0.0139	0.0128	0.0123	0.0104
12	0.0139	0.0128	0.0123	0.0104
13	0.0139	1.0128	0.0123	0.0104
14	0.0667	0.1020	0.0616	0.0688
15	0.2361	0.1122	0.0764	0.1000
16	0.2694	0.2015	0.3596	0.2688
17	0.1833	0.2628	0.2217	0.3042
18	0.1361	0.2219	0.1946	0.1875
19	0.0722	0.0842	0.0714	0.0500
20	0.0278	0.0128	0.0123	0.0167
21	0.0139	0.0128	0.0123	0.0104
>21	0.0139	0.0128	0.0123	0.0104

ALLELE FREQUENCIES (CONTINUED)

FGA

Allele	African American (N=180)	Caucasian (N=196)	Southwest Hispanic (N=203)	Southeast Hispanic (N=191)
<18	0.0139	0.0128	0.0123	0.0131
18	0.0139	0.0306	0.0123	0.0131
18.2	0.0139	0.0128	0.0123	0.0131
19	0.0528	0.0561	0.0788	0.0838
19.2	0.0139	0.0128	0.0123	0.0131
20	0.0722	0.1454	0.0714	0.1178
20.2	0.0139	0.0128	0.0123	0.0131
21	0.1250	0.1735	0.1305	0.1361
21.2	0.0139	0.0128	0.0123	0.0131
22	0.2250	0.1888	0.1773	0.1492
22.2	0.0139	0.0128	0.0123	0.0131
22.3	0.0139	0.0128	0.0123	0.0131
23	0.1250	0.1582	0.1404	0.1492
23.2	0.0139	0.0128	0.0123	0.0131
24	0.1861	0.1378	0.1256	0.1466
24.2	0.0139	0.0128	0.0123	0.0131
24.3	0.0139	0.0128	0.0123	0.0131
25	0.1000	0.0689	0.1379	0.1126
26	0.0361	0.0179	0.0837	0.0471
26.2	0.0139	0.0128	0.0123	0.0131
27	0.0222	0.0128	0.0320	0.0262
28	0.0167	0.0128	0.0123	0.0131
29	0.0139	0.0128	0.0123	0.0131
30	0.0139	0.0128	0.0123	0.0131

ALLELE FREQUENCIES (CONTINUED)

>30	0.0139	0.0128	0.0123	0.0131

D8S1179

Allele	African American (N=180)	Caucasian (N=196)	Southwest Hispanic (N=203)	Southeast Hispanic (N=191
<8	0.0139	0.0128	0.0123	0.0131
8	0.0139	0.0179	0.0123	0.0157
9	0.0139	0.0128	0.0123	0.0131
10	0.0250	0.1020	0.0936	0.0969
11	0.0361	0.0587	0.0616	0.0524
12	0.1083	0.1454	0.1207	0.1073
13	0.2222	0.3393	0.3251	0.3560
14	0.3333	0.2015	0.2463	0.2120
15	0.2139	0.1097	0.1158	0.1204
16	0.0444	0.0128	0.0246	0.0262
17	0.0139	0.0128	0.0123	0.0131
>17	0.0139	0.0128	0.0123	0.0131

ALLELE FREQUENCIES (CONTINUED)

D21S11

Allele	African American (N=179)	Caucasian (N=196)	Southwest Hispanic (N=203)	Southeast Hispanic (N=191)
<24.2	0.0140	0.0128	0.0123	0.0131
24.2	0.0140	0.0128	0.0123	0.0131
24.3	0.0140	0.0128	0.0123	0.0131
26	0.0140	0.0128	0.0123	0.0131
27	0.0615	0.0459	0.0123	0.0183
28	0.2151	0.1658	0.0690	0.1257
29	0.1899	0.1811	0.2044	0.2408
29.2	0.0140	0.0128	0.0123	0.0131
30	0.1788	0.2321	0.3301	0.2382
30.2	0.0140	0.0383	0.0320	0.0340
30.3	0.0140	0.0128	0.0123	0.0131
31	0.0922	0.0714	0.0690	0.0759
31.2	0.0754	0.0995	0.0862	0.0838
32	0.0140	0.0153	0.0123	0.0131
32.1	0.0140	0.0128	0.0123	0.0131
32.2	0.0698	0.1122	0.1355	0.1152
33	0.0140	0.0128	0.0123	0.0131
33.2	0.0335	0.0306	0.0419	0.0367
34	0.0140	0.0128	0.0123	0.0131
34.2	0.0140	0.0128	0.0123	0.0131
35	0.0279	0.0128	0.0123	0.0131
35.2	0.0140	0.0128	0.0123	0.0131
36	0.0140	0.0128	0.0123	0.0131
>36	0.0140	0.0128	0.0123	0.0131

ALLELE FREQUENCIES (CONTINUED)

D18S51

Allele	African American (N=180)	Caucasian (N=196)	Southwest Hispanic (N=203)	Southeast Hispanic (N=191)
<11	0.0139	0.0128	0.0123	0.0131
11	0.0139	0.0128	0.0123	0.0157
12	0.0583	0.1276	0.1059	0.1361
13	0.0556	0.1225	0.1700	0.1178
13.2	0.0139	0.0128	0.0123	0.0131
14	0.0639	0.1735	0.1700	0.1309
14.2	0.0139	0.0128	0.0123	0.0131
15	0.1667	0.1276	0.1379	0.1911
15.2	0.0139	0.0128	0.0123	0.0131
16	0.1889	0.1071	0.1158	0.1414
17	0.1639	0.1556	0.1379	0.1073
18	0.1306	0.0918	0.0517	0.0550
19	0.0778	0.0357	0.0370	0.0471
20	0.0556	0.0255	0.0172	0.0288
21	0.0139	0.0128	0.0197	0.0131
21.2	0.0139	0.0128	0.0123	0.0131
22	0.0139	0.0128	0.0123	0.0131
>22	0.0139	0.0128	0.0123	0.0131

ALLELE FREQUENCIES (CONTINUED)

D5S818

Allele	African American (N=180)	Caucasian (N=195)	Southwest Hispanic (N=203)	Southeast Hispanic (N=240)
<7	0.0139	0.0128	0.0123	0.0104
7	0.0139	0.0128	0.0616	0.0229
8	0.0500	0.0128	0.0123	0.0104
9	0.0139	0.0308	0.0542	0.0500
10	0.0639	0.0487	0.0665	0.0458
11	0.2611	0.4103	0.4212	0.3938
12	0.3556	0.3539	0.2906	0.3167
13	0.2444	0.1462	0.0961	0.1542
14	0.0139	0.0128	0.0123	0.0104
15	0.0139	0.0128	0.0123	0.0104
>15	0.0139	0.0128	0.0123	0.0104

ALLELE FREQUENCIES (CONTINUED)

D13S317

Allele	African American (N=179)	Caucasian (N=196)	Southwest Hispanic (N=203)	Southeast Hispanic (N=240)
<8	0.0140	0.0128	0.0123	0.0104
8	0.0363	0.0995	0.0665	0.1146
9	0.0279	0.0765	0.2192	0.1146
10	0.0503	0.0510	0.1010	0.0771
11	0.2374	0.3189	0.2020	0.3063
12	0.4832	0.3087	0.2168	0.2292
13	0.1257	0.1097	0.1379	0.1083
14	0.0363	0.0357	0.0567	0.0500
15	0.0140	0.0128	0.0123	0.0104
>15	0.0140	0.0128	0.0123	0.0104

ALLELE FREQUENCIES (CONTINUED)

D7S820

Allele	African American (N=210)	Caucasian (N=203)	Southwest Hispanic (N=209)	Southeast Hispanic (N=240)
>6	0.0119	0.0123	0.0120	0.0104
6	0.0119	0.0123	0.0120	0.0104
7	0.0119	0.0172	0.0215	0.0104
8	0.1738	0.1626	0.0981	0.1417
9	0.1571	0.1478	0.0479	0.1250
10	0.3238	0.2906	0.3062	0.2667
11.1	0.0119	0.0123	0.0120	0.0104
11	0.2238	0.2020	0.2895	0.2271
11.3	0.0119	0.0123	0.0120	0.0104
12	0.0905	0.1404	0.1914	0.1875
13	0.0191	0.0296	0.0383	0.0354
14	0.0119	0.0123	0.0120	0.0104
>14	0.0119	0.0123	0.0120	0.0104

ALLELE FREQUENCIES (CONTINUED)

CSF1PO

Allele	African American (N=210)	Caucasian (N=203)	Southwest Hispanic (N=209)	Southeast Hispanic (N=240)
>6	0.0119	0.0123	0.0120	0.0104
6	0.0119	0.0123	0.0120	0.0104
7	0.0429	0.0123	0.0120	0.0104
8	0.0857	0.0123	0.0120	0.0104
9	0.0333	0.0197	0.0120	0.0125
10	0.2714	0.2537	0.2536	0.2542
10.3	0.0119	0.0123	0.0120	0.0104
11	0.2048	0.3005	0.2656	0.2958
12	0.3000	0.3251	0.3923	0.3563
12.1	0.0119	0.0123	0.0120	0.0104
13	0.0548	0.0714	0.0646	0.0688
14	0.0119	0.0148	0.0120	0.0104
15	0.0119	0.0123	0.0120	0.0104
>15	0.0119	0.0123	0.0120	0.0104

ALLELE FREQUENCIES (CONTINUED)

TPOX

Allele	African American (N=209)	Caucasian (N=203)	Southwest Hispanic (N=209)	Southeast Hispanic (N=240)
>6	0.0120	0.0123	0.0120	0.0104
6	0.0861	0.0123	0.0120	0.0104
7	0.0215	0.0123	0.0120	0.0104
8	0.3684	0.5443	0.5550	0.5063
9	0.1818	0.1232	0.0335	0.0833
10	0.0933	0.0370	0.0335	0.0625
11	0.2249	0.2537	0.2727	0.2771
12	0.0239	0.0394	0.0933	0.0646
13	0.0120	0.0123	0.0120	0.0104
>13	0.0120	0.0123	0.0120	0.0104

TH01

Allele	African American (N=210)	Caucasian (N=203)	Southwest Hispanic (N=209)	Southeast Hispanic (N=240)
>5	0.0119	0.0123	0.0120	0.0104
5	0.0119	0.0123	0.0120	0.0104
6	0.1095	0.2266	0.2321	0.2125
7	0.4405	0.1724	0.3373	0.2521
8	0.1857	0.1256	0.0813	0.1042
8.3	0.0119	0.0123	0.0120	0.0104
9	0.1452	0.1650	0.1029	0.1854
9.3	0.1048	0.3054	0.2416	0.2354
10	0.0143	0.0123	0.0120	0.0104

ALLELE FREQUENCIES (CONTINUED)

>10	0.0119	0.0123	0.0120	0.0104

D16S539

Allele	African American (N=209)	Caucasian (N=202)	Southwest Hispanic (N=208)	Southeast Hispanic (N=240)
>8	0.0120	0.0124	0.0120	0.0104
8	0.0359	0.0198	0.0168	0.0229
9	0.1986	0.1040	0.0793	0.1458
10	0.1101	0.0668	0.1731	0.0958
11	0.2943	0.2723	0.3149	0.2813
12	0.1866	0.3391	0.2861	0.2542
13	0.1651	0.1634	0.1034	0.1625
14	0.0120	0.0322	0.0240	0.0354
15	0.0120	0.0124	0.0120	0.0104
>15	0.0120	0.0124	0.0120	0.0104

WORKSHEETS

APPENDIX E

DNA QUANTITATION (MODEL)

Case Number(s) _____ Analyst _____

_____ Date _____

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						

Unless otherwise stated, 5 μl of the DNA standard was added to the tube. ND = Not detected. > = Greater than. < = Less than.

Spotting Solution		QuantiBlot Wash Solution	
Biodyne B Membrane		1X Citrate Buffer	
QuantiBlot Kit		30% Hydrogen Peroxide	
PreWetting Solution		3% Hydrogen Peroxide	
Hybridization Solution		Tetramethyl Benzidine	

APPENDIX F

DNA SUMMARY (MODEL)

EXHIBIT	DQA1	LDLR	GYPA	HBGG	D7S8	GC	D1S80

APPENDIX G

AMPLIFICATION DATA (MODEL)

Analyst _____ Extraction date: _____

Case #	Item #	QB LOT # _____ CONTROL DATE _____ Ng/μl	PM + DQA1 LOT # _____ CONTROL DATE _____ Thermocycler _____ Date amp. _____ μl DNA	WELL

APPENDIX H

FREQUENCIES (MODEL)

Case Number: _____

Date: _____

Analyst: _____

	African American	Caucasian	Southwestern Hispanics
DQA1			
LDLR			
GYPA			
HBGG			
D7S8			
GC			
D1S80			
TOTAL			

APPENDIX I

STR DILUTION AND AMPLIFICATION WORKSHEET (MODEL)

			QuantiBlot		Dilution		Profiler Plus	Cofiler
			Lot #				Lot #	Lot #
			Exp		TE Lot #		Exp	Exp
	Analyst		Date		Date		Therm	Therm
							Date	Date
	Case #	Item #	ng/µl		DNA (µl)	TE (µl)	Well	Well
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
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19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
32								
33								
34								
35								

Total # Samples

+1

RXN Mix

X 21

TAQ

X1

PRIMERS

X11

APPENDIX J

GENESCAN WORKSHEET (MODEL)

CASE # _____ **FILE #** _____

ANALYST _____

IDENTIFIER	SIZE	PEAK HEIGHT	DATA POINT	COMMENTS	Delete

OLA = Off Ladder Allele; PU = Pull Up; B =Blue; G = Green; Y = Yellow; R = Red; DP = Dye Peaks
M = manual delete A = computer delete; D - deleted run from Genotyper, -A = incomplete A addition
RB - Reagent Blank, RBS = Reagent Blank Sperm, RBE = Reagent Blank Epithelial, NAB = Negative Amplification
CECT - Cut Extraction Control Tissue, CECS - Cut Extraction Control Sperm, CECE = Cut Extraction Control Epithelial
PC - Positive amplification Control

APPENDIX K

MIXTURE ANALYSIS WORKSHEET – PROFILER PLUS (MODEL)

Case # _____ Item # _____ Analyst _____ Date _____

Locus	Allele	Peak Height	Possible component profiles giving rise to observed mixed profiles		Interpretation and Comments
			A (major)	B (minor)	
D3S1358					
vWA					
FGA					
AMELOG					
D8S1179					
D21S11					
D18S51					
D5S818					
D13S317					
D7S820					

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DNA ANALYSIS SOP, VERSION 2.1
JUNE 2002

APPENDIX L

MIXTURE ANALYSIS WORKSHEET – COFILER (MODEL)

Case # _____ Item # _____ Analyst _____ Date _____

Locus	Allele	Peak Height	Possible component profiles giving rise to observed mixed profiles		Interpretation and Comments
			A (major)	B (minor)	
D3S1358					
D16S539					
AMELOG					
THO1					
TPOX					
CSF1PO					
D7S820					

APPENDIX M

DNA STOCK SOLUTIONS LOG – 8 μG/μL BOVINE SERUM ALBUMIN (BSA) (MODEL)

Solution Preparation:

Before starting, UV treat all equipment and glassware.

Add 8 g BSA per every 1L (0.8 g per every 100 ml) autoclaved ultrapure H_2O. Mix thoroughly. Sterile filter through a Nalgene 0.2 or 0.45 micron filter unit. Aliquot into sterile 15 ml tubes. Aliquot one 15 ml tube into sterile 1.5 ml Sarstedt tubes as needed. Store at -20°C.

Date Prepared	Amount	BSA Lot #	Preparer

(BSA2)

APPENDIX N

DNA STOCK SOLUTIONS LOG – 10X CITRATE BUFFER (MODEL)

(1M SODIUM CITRATE, PH 5.0) (2 L)

Solution Preparation:

Dissolve 368 g trisodium citrate, dihydrate ($Na_3C_6H_5O_7 \bullet 2H_2O$) in 1600 ml ultrapure water. Adjust pH to 5.0 (± 0.2) using approximately 60 g citric acid monohydrate ($C_6H_8O_7 \bullet H_2O$). Bring up to 2 liter with ultrapure water, mix, and autoclave.

Date Prepared	Amount	$Na_3C_6H_5O_7 \cdot 2 H_2O$ lot#	$C_6H_8O_7 \cdot H_2O$ lot#	pH	Preparer

(10CITBUF)

APPENDIX O

DNA STOCK SOLUTIONS LOG – 0.5M EDTA, PH 8.0 (MODEL)

Solution Preparation:

Slowly add 186.1 g disodium ethylenediaminetetraacetic acid-dihydrate ($Na_2EDTA \cdot 2H_2O$) to 800 ml ultrapure water. Stir vigorously on a magnetic stirrer. Adjust the pH to 8 by adding NaOH pellets (approximately 20 g). Check pH and use 10 N NaOH if needed for small pH adjustments. **Note: EDTA will not go into solution without adjusting the pH.** Bring to 1 liter with ultrapure water. Sterilize by autoclaving. Store at room temperature.

Date Prepared	Amount	$Na_2EDTA \cdot 2H_2O$ lot#	NaOH pellets lot#	10 N NaOH lot#	pH	Preparer

(EDTA2)

APPENDIX P

DNA STOCK SOLUTIONS LOG – 5M NACL (MODEL)

Solution Preparation:

Dissolve 292 g NaCl in 800 ml ultrapure water. Adjust the final volume to 1 liter. Sterilize by autoclaving. Store at room temperature.

Date Prepared	Amount	NaCl (Reagent Grade)/ lot#	Preparer

NACL2

APPENDIX Q

DNA STOCK SOLUTIONS LOG – PBS BUFFER, PH 7.4 (PHOSPHATE BUFFER SALINE) (MODEL)

[2.7MM KCL, 137MM NACL, 1.5MM KH_2PO_4 , PH 7.4 (1L)]

Solution Preparation:

Dissolve 0.2 g KCl, 8.0 g NaCl, 0.2 g KH_2PO_4, 1.1 g of Na_2HPO_4 , anhydrous) in 800 ml ultrapure water. Adjust pH of solution to 7.4, if necessary. Adjust to final volume to 1 liter using ultrapure water. Sterilize by autoclaving. Store at room temperature.

Date Prepared	Amount	KCl lot#	NaCl lot#	KH_2PO_4 lot#	$Na_2HPO_4 \bullet H_2O$ lot#	Preparer	Verified by: initials/date

PBSBUFF

APPENDIX R

DNA STOCK SOLUTIONS LOG – 1M SODIUM ACETATE, PH 5.2 (MODEL)

100 ML

Solution Preparation:

Dissolve 13.6 g $CH_3COONa \cdot 3H_2O$ (sodium acetate) in 80 ml ultrapure water. Adjust pH to 5.2 by adding glacial acetic acid (approximately 2 ml). Adjust the final volume to 100 ml. Sterilize by autoclaving. Store at room temperature.

Date Prepared	Amount	Sodium Acetate lot#	Glacial Acetic lot#	Preparer

NAOAC

APPENDIX S

DNA STOCK SOLUTIONS LOG – 20% (W/V) SODIUM DODECYL SULFATE (MODEL) (SDS) (1L)

Solution Preparation:

Warning: SDS is an irritant. Avoid skin contact and inhalation.

Slowly dissolve 200 g electrophoresis grade/ultrapure SDS in 800 ml ultrapure H_2O. May need to warm solution (i.e., 50ºC incubator) to dissolve the SDS. Adjust to 1 liter using ultrapure water. Mix thoroughly.

Date Prepared	Amount	SDS Lot #	Preparer

(20%SDS2)

APPENDIX T

DNA STOCK SOLUTIONS LOG – 20X SSPE BUFFER (MODEL)

(3.6M NACL, 200MM $NaH_2PO_4 \bullet H_2O$, 20MM EDTA, PH 7.4) 2L

Solution Preparation:

In 1600 ml ultrapure water dissolve 14.8 g $Na_2EDTA \bullet 2H_2O$. Adjust pH to 6 with 10 N NaOH. Add 420 g sodium chloride (NaCl) and 55.2 g sodium phosphate, monobasic, monohydrate ($NaH_2PO_4 \bullet H_2O$). Adjust pH to 7.4 using 10 N NaOH (about 10 ml). Bring up to 2 liters, and mix. Autoclave.

Date Prepared	Amount	NaCl lot#	$NaH_2PO_4 \bullet H_2O$ lot#	$Na_2EDTA \bullet 2H_2O$ lot#	10N NaOH lot#	pH	Preparer

(20XSSPE)

APPENDIX U

DNA STOCK SOLUTIONS LOG – 10X TBE (MODEL)

890MM TRIS, 890MM BORIC ACID, 20MM EDTA (1L)

Solution Preparation:

To approximately 800 ml ultrapure H_2O add 40 ml 0.5M EDTA pH8.0. Add 108 g Tris base and 55 g Boric acid to the diluted EDTA solution. Stir until dissolved. Adjust volume to 1L with ultrapure water. Filter through a Nalgene 0.2 or 0.45 micron filter unit and store at room temperature in clear container.

Note: If a white precipitate is noted in 10X TBE, the buffer should be discarded and remade.

Date Prepared	Amount	0.5 M EDTA lot#	Tris base lot#	Boric acid lot#	Preparer

(10XTBE2)

APPENDIX V

DNA STOCK SOLUTIONS LOG – 1M TRIS HCL, PH 7.5 (MODEL)

Note: Use an electrode suitable for measuring Tris buffers. Adjust volume to 1 liter with ultrapure water. Sterilize by autoclaving. Store at room temperature.

Solution Preparation:

Dissolve 121.1 g Tris base in 800 ml ultrapure water. Adjust pH to 7.5 (± 0.2) using concentrated HCl (approximately 65 ml).

Date Prepared	Amount	Tris Base lot#	HCl, concentrated lot#	Preparer	pH

TRISPH75

APPENDIX W

DNA STOCK SOLUTIONS LOG – 1M TRIS HCL, PH 8.0 (MODEL)

Note: Use an electrode suitable for measuring Tris buffers. Adjust volume to 1 liter with ultrapure water. Sterilize by autoclaving. Store at RT.

Solution Preparation:

Dissolve 121.1 g Tris base in 800 ml ultrapure water. Adjust pH to 8.0 (± 0.2) using concentrated HCl (approximately 45 ml).

Date Prepared	Amount	Tris Base lot#	HCl, concentrated lot#	Preparer	pH

(TRISPH82)

APPENDIX X

DNA WORKING SOLUTIONS LOG – 5% (W/V) CHELEX™ SOLUTION (MODEL)

Solution Preparation:

Before starting, UV treat all equipment and glassware. When pipetting Chelex™ Stock Solutions, the resin beads must be distributed evenly in solution; this can be achieved by gently mixing with a stir bar in a beaker. Also, the pipette tip used must have a relatively large bore; 1ml tips are adequate.

Add 5 g Chelex™ per every 100 ml sterile ultrapure water. Aliquot into sterile 1.5 ml tubes. Check pH of one of the tubes, it should be greater than 9.

Date Prepared	Amount	Chelex™ lot#	Preparer	Verified by: initials/date

CHELEX5

APPENDIX Y

DNA WORKING SOLUTIONS LOG – 20% (W/V) CHELEX™ SOLUTION (MODEL)

Solution Preparation:

Before starting UV treat all equipment and glassware. When pipetting Chelex™ Stock Solutions, the resin beads must be distributed evenly in solution; this can be achieved by gently mixing with a stir bar in a beaker. Also, the pipette tip used must have a relatively large bore; 1ml tips are adequate.

Add 20 g Chelex™ per every 100 ml sterile ultrapure water. Aliquot into sterile 1.5 ml tubes. Check pH of one of the tubes, it should be greater than 9.

Date Prepared	Amount	Chelex™ lot#	Preparer	Verified by: initials/date

CHELEX20

APPENDIX Z

DNA WORKING SOLUTIONS LOG – DEIONIZED FORMAMIDE (MODEL)

50 CT

Solution Preparation:

One of the following two methods can be employed.

1. Aliquot Hi-Di Formamide (ABI Cat. #4311320) into microcentrifuge tubes. Store at -20ºC. Use only if lot freezes.

2. Mix 50 ml Formamide and 5 g Dowex MR3™ (Sigma Cat #I9005) ion exchange resin. Stir for 30 minutes at room temperature. Check that the pH is greater than 7. If the pH is less than 7, start the process again utilizing the same formamide but different Dowex MR3™ ion exchange resin. Allow the resin to settle. Filter the formamide and aliquot into 1.5 ml vials. Store up to 3 months at -20°C

Date Preparation	Amount	Formamide lot#	Dowex MR3™ resin lot	Preparer

(FORMAMIDE)

APPENDIX AA

DNA WORKING SOLUTIONS LOG – DIGEST BUFFER (MODEL)

[10MM TRIS-HCL, 10MM EDTA, 50MM NACL, 2% SDS, PH 7.5 (100 ML)]

Solution Preparation:

UV treat all glassware and equipment. Mix together 1ml 1M Tris-HCl, pH 7.5; 2 ml 0.5M EDTA; 1 ml 5M NaCl; 10 ml 20% (w/v) SDS; and 86 ml sterile ultrapure water. Aliquot into 50 ml conical tubes. Store at room temperature.

Date Prepared	Amount	1 M Tris-HCl lot #	0.5 M EDTA lot #	5 M NaCl lot #	20% SDS lot #	Preparer	Verified by: initials/date

DIGBUFF

APPENDIX AB

DNA WORKING SOLUTIONS LOG – 1M DTT (DITHIOTHREITOL), 10MM SODIUM ACETATE, PH 5.2 (MODEL)

5 ML

Solution Preparation:

Dissolve 0.77 g dithiothreitol in 5 ml sterile ultrapure water. Add 50 μL 1M sodium acetate pH 5.2. Solution may be sterilized by filtration through a 0.2-0.45 micron filter. Do not autoclave. Aliquot (recommended 0.5 ml) and store at -20°C.

Date Prepared	Amount	Dithiothreitol lot#	1 M sodium acetate, pH 5.2 lot #	Preparer	Verified by: initials/date

DTT

APPENDIX AC

DNA WORKING SOLUTIONS LOG – 200MM EDTA (MODEL)

10 ML

Solution Preparation:

Add 4.0 ml 0.5M EDTA to 6.0 ml ultrapure water and mix thoroughly. The solution may be divided into 300 μl aliquots using microcentrifuge tubes.

Date Prepared	Amount	0.5M EDTA lot#	Preparer

(200MEDTA)

APPENDIX AD

DNA WORKING SOLUTIONS LOG – 3% HYDROGEN PEROXIDE (MODEL)

Solution Preparation:

Add 1ml 30% H_2O_2 to 9ml sterile ultrapure water and vortex to mix. The solution may be divided into 400 µl aliquots using microcentrifuge tubes. Protect from light. Store at 2° to 8°C.

Note: 3% Hydrogen Peroxide expires 1 month after preparation.

Date Prepared	Amount	30% H_2O_2 lot #	Preparer

(3%H202)

APPENDIX AE

DNA WORKING SOLUTIONS LOG – 30% HYDROGEN PEROXIDE (MODEL)

Solution Preparation:

Aliquot 5 ml 30% H_2O_2 into 15 ml tubes and store at 4ºC in area where QuantiBlot® is performed.

Date Prepared	Amount/# of tubes	30%H_2O_2 lot #	Preparer

(30%H_20_2)

APPENDIX AF

DNA WORKING SOLUTIONS LOG – ORGANIC SPERM WASH BUFFER (MODEL)

(10MM TRIS-HCL – 10MM EDTA – 50 MM NACL – 2 % SDS, PH 8.0, 500 ML)

Solution Preparation:

Add 5 ml 1M Tris-HCl, (pH 8.0), 10 ml 0.5M EDTA (pH 8.0 ± 0.05), 5 ml 5M NaCl (or use 0.29 g NaCl), and 50 ml 20% SDS to 430 ml ultrapure water and adjust volume to 500 ml. Check pH. Autoclave. Store at room temperature.

Date Prepared	Amount	1M Tris-HCl, pH 8.0 lot #	0.5M EDTA lot#	5M NaCl lot#	20% SDS lot#	pH	Preparer Verified/date

ORGANIC SPERM WASH BUFFER

APPENDIX AG

DNA WORKING SOLUTIONS LOG – ORGANIC STAIN EXTRACTION BUFFER (MODEL)

(10MM TRIS – 100MM NACL – 10MM EDTA – 2% SDS, 1L)

Solution Preparation:

Dissolve 5.84 g NaCl in 500 ml ultrapure water by stirring. To this solution add 10 ml 1M Tris (pH 8.0 ± 0.05), 20 ml 0.5 M EDTA, and 100 ml 20% SDS. Titrate to pH 8.0 with HCl. Bring to a final volume of 1L with ultrapure water. Autoclave. Store at room temperature.

Date Prepared	Amount	NaCl Lot #	1M Tris, pH 8.0 lot #	0.5M EDTA lot #	20% SDS lot #	pH	Preparer	Verified/date

ORGANIC STAIN EXTRACTION BUFFER

APPENDIX AH

DNA WORKING SOLUTIONS LOG – PM/DQA1 WASH SOLUTION (MODEL)

[2.5X SSPE, 0.1% w/v SDS (2L)]

Solution Preparation:

Add 250 ml 20X SSPE and 10 ml 20% w/v SDS to 1740 ml ultrapure water and mix thoroughly.

Note: Wash solution solids must be in solution before use; warming (e.g., 50°C incubator) may be required to dissolve solids completely. Preparation in a clear glass container is recommended to facilitate visual inspection for solids during warming.

Date Prepared	Amount	20X SSPE lot #	20% SDS lot #	Preparer

PMDQAwash

APPENDIX AI

DNA WORKING SOLUTIONS LOG – PROTEINASE K 10 MG/ML (MODEL)

10 ML

Solution Preparation:

Dissolve 100 mg Proteinase K in 10 ml sterile ultrapure water. Aliquot solution (0.5 ml recommended) and store frozen at -20°C.

Date Prepared	Amount	Proteinase K lot#	Preparer	Verified by: initials/date

PROK

A APPENDIX AJ

DNA WORKING SOLUTIONS LOG – QUANTIBLOT® PREWETTING SOLUTION (MODEL)

[0.4N NAOH, 25MM EDTA, (500 ML)]

Solution Preparation:

Add 20 ml 10N NaOH, 25 ml 0.5M EDTA, to 455 ml ultrapure water and mix thoroughly.

Date Prepared	Amount	10 N NaOH lot #	0.5 M EDTA lot #	Preparer

QBPREWET

APPENDIX AK

DNA WORKING SOLUTIONS LOG – QUANTIBLOT® SPOTTING SOLUTION (MODEL)

[0.4N NAOH, 25MM EDTA, 0.00008% BROMOTHYMOL BLUE (75 ML)]

Solution Preparation:

Add 3ml 10N NaOH, 3.75 ml 0.5M EDTA, and 150 μL 0.04% Bromothymol Blue (provided in kit) to 68 ml ultrapure water and mix thoroughly. Note: Spotting solution is stable for at least three months at room temperature.

Date Prepared	AMOUNT	10N NaOH lot #	0.5M EDTA lot #	0.04% Bromo. Blue lot #	Preparer

QBSPOT

APPENDIX AL

DNA WORKING SOLUTIONS LOG – QUANTIBLOT WASH SOLUTION (MODEL)

[1.5X SSPE, 0.5% W/V SDS (2L)]

Solution Preparation:

Add 150 ml 20X SSPE and 50 ml 20% w/v SDS to 1800 ml ultrapure water and mix thoroughly.

Note: Wash solution solids must be in solution before use; warming (e.g., 50°C incubator) may be required to dissolve solids completely. Preparation in a clear glass container is recommended to facilitate visual inspection for solids during warming.

Date Prepared	Amount	20X SSPE lot #	20% SDS lot #	Preparer

QBWASH

APPENDIX AM

DNA WORKING SOLUTIONS LOG QUANTIBLOT/PM +DQA1 HYBRIDIZATION SOLUTION (MODEL)

[5X SSPE, 0.5% w/v SDS (1L)]

Solution Preparation:

Add 250 ml 20X SSPE and 25 ml 20% SDS w/v to 725 ml ultrapure water and mix thoroughly.

Note: Hybridization solution solids must be in solution before use; warming (e.g., in 50^{o}C incubator) may be required to dissolve solids completely. Preparation in a clear glass container is recommended to facilitate visual inspection for solids during warming.

Date Prepared	Amount	20X SSPE lot #	20% SDS	Preparer

HYBSOLN

APPENDIX AN

DNA WORKING SOLUTIONS LOG – 20% SARKOSYL (MODEL)

(N-LAURYLSARCOSINE, 100 ML)

Solution Preparation:

Add 20 g N-laurylsarcosine ($C_{15}H_{28}NO_3Na$) to ultrapure water and stir until dissolved. Bring to a final volume of 100 ml with ultrapure water and sterilize by passage through a 0.45 μ sterile filter.

Date Prepared	AMOUNT	N-laurylsarcosine lot#	Preparer	Verified by: initials/date

20% SARKOSYL

APPENDIX AO

DNA WORKING SOLUTIONS LOG – TE BUFFER (MODEL)

[10MM TRIS-HCL, 0.1MM EDTA, PH 8.0 (1L)]

Solution Preparation:

Mix together 10 ml 1M Tris-HCl, pH 8.0, 0.2 ml 0.5M EDTA and 990 ml ultrapure water and sterilize by autoclaving. Store at room temperature.

Date Prepared	Amount	Tris-HCl, pH 8.0 lot #	0.5 M EDTA lot #	Preparer	Verified by: initials/date

TEBUFF

APPENDIX AP

DNA WORKING SOLUTIONS LOG – TRIS/EDTA/NACL (MODEL)

(10MM TRIS-HCL – 100MM NACL – 1MM EDTA, PH 8.0, 100 ML)

Solution Preparation:

Add 1ml 1M Tris-HCl to approximately 75 ml ultrapure water. To this solution add 0.584 g NaCl and 200 µl 0.5M EDTA. Stir until dissolved. Adjust the pH to 8.0 with 1N NaOH and bring to a final volume of 100 ml with ultrapure water. Autoclave and store at room temperature.

Date Prepared	Amount	NaCl lot #	1M Tris, pH 8.0 lot #	0.5 M EDTA lot #	1N NaOH lot #	pH	Preparer	Verified/date

TRIS/ETDA/NACL

APPENDIX AQ

DNA CHEMICAL LOG (MODEL)

Chemical/Reagent	Supplier	Catalog #	Lot #	Received	Expiration Date

APPENDIX AR

DNA QA/QC FORM (MODEL)

Commercial Kit:

_____

_____

_____

Purchased From:

_____

_____

_____

DATE RECEIVED	LOT #	EXP. DATE	DATE OPENED	VERIFIED BY/DATE

DNA QAQC FORM

APPENDIX AS

EXTRACTION SUMMARY SHEET (MODEL)

Analyst _____ **Date** _____

	Case #	Item #	Description	Concentrate Resulting Volume (µL)	Comments	Reagent	Lot #
1						TE -4	
2						5% Chelex™	
3						PBS	
4						Proteinase K	
5						20% Chelex™	
6						Digest Buffer	
7						1 M DTT	
8						Tris/EDTA/NaCl	
9						20% Sarkosyl	
10						Org. Sp. Wash	
11						Saline	
12							
13							
14							
15							
16							
17							
18							
19							
20							

CEC = Cut Extract Control: E = Epithelial Fraction, S = Sperm fraction, T = Tissue

Org. Sp. Wash = Organic Sperm Wash

EXTRACTION SUMMARY SHEET

APPENDIX AT

CBI DNA DATABASE SAMPLE EXTRACTION/ AMPLIFICATION WORKSHEET (MODEL)

Identifiler

1. Stain Cards Spotted: INT
2. Samples Cut: _____ INT _____ Identifiler kit lot # _____
3. Extraction Method: CHELEX or FTA DATE _____ INT _____
4. LOT: TE _____ WASH _____ CHELEX _____ Expiration date _____
5. Detection Date/Instrument _____ Thermocycler used _____
6. AA's Submitted: _____
7. CODIS ENTRY: _____

	Sample Info				ul DNA		Well #		
	PCR	CBI #	LAST NAME	OCA					
1									
2									total #
3									of
4									samples
5									
6									
7									
8									RXN MIX
9									
10									X 10.5 ul
11									
12									
13									
14									
15									TAQ
16									
17									X 0.5 ul
18									
19									
20									
21									
22									
23									Primers
24									
25									X 5.5 ul
26									

APPENDIX AU

GENESCAN/GENOTYPER REVIEW SHEET (MODEL)

Project: _____ Runs: _____ Size Std: _____ Analysis Param: _____ Reviewed By: _____

Sample #	Well #	Problem	GS Action	GT Action	Comments
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			
		Pull-Up OLA Spike _____			

OLA= off ladder allele G=Green B=Blue R=Red Y=Yellow O= Orange pk=peak pks=peaks bp= basepair sz=size std=standard Param.=parameters
PH=peak height GS=Genescan GT=Genotyper ID= Identifiler CO=Cofiler PP=ProfilerPlus -A=A addition incomplete
D3=D3S1358 D5=D5S818 CSF=CSF1PO D7=D7S820 THO=THO1 D13=D13S317 D16=D16S539 D18=D18S51 D21=D21S11 AMEL= amelogenin
D8=D8S1179 D19=D19S433 D2=D2S1338 P/U=pullup st=stutter ↑ =increase ↓ =decrease spk=spike SO=spill over
RB=reagent blank NAB=negative amplification blank PC=positive control

APPENDIX AV

3200 LOAD SHEET (MODEL)

Plate Name:						
	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						
	7	8	9	10	11	12
A						
B						
C						
D						
E						
F						
G						
H						